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PLENARY

Disease resistance in cultivated sunflower derived from public germplasm collections

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ABSTRACT

The incorporation of disease resistance into cultivated sunflower has been a major goal of private and public plant breeders. With several dozen fungal pathogens and a few bacterial and viral problems, the primary means of managing sunflower diseases has been through genetic resistance. While the ultimate source of all new genes in cultivated sunflower is the wild *Helianthus* species gene pool, the diverse collections of cultivated sunflower maintained by several governmental research entities have been a major resource for improved disease resistance. The two largest sunflower germplasm collections in the world are maintained by the USDA-National Plant Germplasm System (NPGS) in Ames, IA, U.S.A. and by the N. I. Vavilov Research Institute of Plant Industry (VIR) in St. Petersburg, Russia. The USDA collection currently numbers over 1700 cultivated accessions (Plant Introductions, or PIs), originating from 59 countries, while the VIR collection has 2300 cultivated accessions, originating from 48 countries. Data on disease resistance of the USDA collection is publically available via the Internet at http://www.ars-grin.gov/npgs/acc/acc_queries.html, and seed is available free of charge. Seeds from the VIR collection are likewise available to researchers, with disease resistance data available upon request. Historically, the USDA collection has been used extensively by the USDA Sunflower Research Unit in Fargo, North Dakota with many of its germplasm releases derived from Plant Introductions. Resistance to downy mildew, rust, Sclerotinia stalk rot and head rot, Phomopsis stem canker, and Verticillium wilt have been identified in multiple USDA Plant Introductions. These PIs have been used directly to develop inbred lines or populations released specifically for such disease resistance. The USDA cultivated sunflower collection is also used by private and other public breeders within and outside of the U.S., but specific use of the Plant Introductions is not always documented. Through continued additions to these public germplasm collections, the genetic diversity of sunflower (and of disease resistance genes) will be expanded, and the free exchange of this germplasm will benefit both public and private researchers.

INTRODUCTION

The normal source of disease resistance genes for the improvement of any crop is cultivated germplasm, and one of the most diverse sources of germplasm are the publically maintained seed collections supported by several national governments. For sunflower, these include seed collections maintained by Argentina (INTA), Brazil (Embrapa), Canada (Agriculture and Agri-Food Canada; http://pgrc3.agr.gc.ca/cgi-bin/npgs/html/crop.pl?79), China (CGRIS), France (INRA), India (NBPGR), Russia (VIR), Serbia (Institute of Oilseed Crops, Novi Sad), Spain (CSIC),the U.S.A. (USDA-NPGS) and there are likely others. Many of these germplasm collections are relatively small, some are no longer publically funded, and most do not freely distribute seeds, leaving the USDA-NPGS and VIR as the two major public sources of sunflower germplasm in the world.

USDA-NPGS Sunflower Collection: The USDA sunflower germplasm collection is maintained by the USDA North Central Regional Plant Introduction Station, located at Ames, Iowa, which is part of the National Plant Germplasm System (NPGS). The collection currently consists of 1713 cultivated accessions (97% available for distribution) and 2236 accessions of wild annual and perennial *Helianthus* species (Marek et al., 2008). Within the cultivated accessions, the 'improvement status' lists cultivars (21%), inbred lines (25%), populations (10%), landraces (6%) and unclassified (38%). The cultivated accessions originated (or were donated from) 58 countries, with one-half originating from four countries: U.S.A. (28%), Russia (13%), Turkey (7%), and Spain (7%), and another seven countries (Argentina,

Canada, Germany, Hungary, Iran, Romania, and Serbia) comprising another quarter of the collection. Information on both the cultivated sunflower and wild *Helianthus* accessions in the collection is found online in the Germplasm Resources Information Network (GRIN) at: http://www.ars-grin.gov/cgi-bin/npgs/html/crop.pl?79. Evaluations of the accessions on a variety of traits are made primarily USDA and university scientists, but any researcher obtaining accessions is requested to share their data for inclusion in the GRIN system. Traits (or descriptors) listed in GRIN are grouped into categories of chemical, cytological, disease, growth, insect, molecular, morphology and few minor ones. Within the 'disease' category, there are 28 descriptors for diseases ranging from *Albugo* (white rust) to Verticillium wilt (http://www.ars-grin.gov/cgi-bin/npgs/html/desclist.pl?79) but only 21 categories have data listed in GRIN. Diseases for which there is the most data available include rust (with 2268 data entries), downy mildew (1770), Sclerotinia stalk rot (1265), Phomopsis stem canker (1106), and *Albugo* (1059). Of the 8870 data entries for various disease evaluations, the senior author has contributed 88% of the total over the last twenty years (http://www.ars-grin.gov/cgi-bin/npgs/html/coop.pl?74750). To view disease evaluation data on the USDA sunflower collection, one can either look up a specific accession (http://www.ars-grin.gov/npgs/acc/acc_queries.html) or can view a disease descriptor and see the entire dataset (i.e, Sclerotinia evaluations from 1986: http://www.ars-grin.gov/cgi-bin/npgs/html/eval.pl?170).

Seeds of both the cultivated and wild *Helianthus* accessions are freely available to researchers in all but a few countries (blocked by the U.S. State Dept.) by writing to the sunflower curator (Dr. Laura Marek, address listed in the title) or online at: http://www.ars-grin.gov/npgs/orders.html . In the last ten years, an average of 56 requests/year were made for cultivated sunflowers, coming from 38 countries, with an average request entailing 17 accessions. During the last five years there has been a 20% increase in the number of requests, reflecting the world-wide interest in the USDA germplasm collection. Since the USDA collection is quite large, and information is not available on all diseases on all accessions, a 'core collection' was created to enable researchers to obtain a representative cross section of the entire collection (Brothers and Miller, 1999). This 'core collection' consists of 112 entries from 38 countries, and while it represents only 6.5% of the cultivated collection when formulated, it was statistically selected to represent the range of traits across the entire collection.

VIR germplasm collection. The Vavilov Research Institute of Plant Industry (VIR), headquartered in St. Petersburg, Russia, is the largest germplasm repository in the world. Begun in 1894, its mission includes collection, conservation and study of all crop plants, of which sunflower is but one of the 425 genera in the collection. Sunflower research and curation is supervised by Dr. Vera Gavrilova, head of the Oil and Fiber Crops Genetics Resources Dept. (http://www.vir.nw.ru/contact.htm). Seed propagation of cultivated sunflower and most wild *Helianthus* species is done at the Kuban station, one of the 12 satellite stations (http://www.vir.nw.ru/structure.htm), located in Botanika, about one hour east of Krasnodar. The VIR sunflower collection currently consists of ~ 2300 cultivated accessions. Seed requests should be sent to Dr. Galena Filipenko (g.filipenko@vir.nw.ru) head of the gene bank.

Web-accessible data on the VIR sunflower collection is available at http://www.vir.nw.ru/data/dbf.htm , but is limited to variables such as accession name and country of origin. For specific information on disease resistance or other characters, one must contact Dr. Gavrilova directly. The VIR cultivated sunflower collection has been partially tested for resistance to several diseases, most notably Phomopsis stem canker (1860 accessions tested), Sclerotinia head rot and stalk rot, downy mildew (multiple races), and the parasitic weed *Orobanche*.

The objectives of this paper are to highlight the identification of cultivated sunflowers, available from the USDA and VIR collections, with resistance to a few of the most serious diseases affecting sunflower worldwide.

MATERIALS AND METHODS

Disease assessment protocols: Ideally, a standardized inoculation and evaluation protocol should be used to assess disease resistance, as this will make the results both easier to understand and applicable to other environments. An attempt was made to collate such methodology by the FAO Research Network on Sunflower (Ilescu, 1995), in which methods are listed for nine diseases and broomrape. In the intervening years, many additional artificial inoculation methods and rating systems have been proposed for several sunflower pathogens, most notably *Phomopsis* and *Sclerotinia* (Degener et al., 1999; Hahn, 2000; Langar et al., 2002; Masirevic et al., 1988; Viguie et al., 1999), but there has been no subsequent, single publication in which these methods have been collated. The disease inoculation/evaluation methods listed below for USDA and VIR tests illustrate a few of the methods used for two diseases.

VIR- *Phomopsis*. Phomopsis evaluations were done on VIR accessions planted at the Kuban Experiment Station in years when the environment was conducive for natural infection. Approximately 25% of the VIR collection is planted annually for seed increases at Botanika, and in 2000, 2002, 2004, 2008 and 2009, the level of Phomopsis infection on the reference inbred line BK-71 was high enough (95-100% infection) to warrant taking Phomopsis notes. Each entry for seed increase is planted in a single, three-row block, with a total of 42 plants/plot. From 1997 to 2000 the cultivar Peredovik was used as a reference check, but has been replaced by the cultivar Master since 2001. Resistance assessments start in August and are made three times at 10-day intervals, with disease incidence calculated as the percentage of injured plants in the three-row plot. Those accessions showing high levels of resistance may have a second Phomopsis rating if a subsequent year's increase had *Phomopsis*, or in some cases the accession was tested with artificial inoculations by VNIIMK personnel in Krasnodar.

USDA- *Phomopsis.* Phomopsis stem canker has been an intermittent disease threat in the U.S.(Gulya, 2002) and there has been some effort to screen the USDA cultivated sunflower collection. In 1989, 499 USDA Plant Introductions were evaluated in Novi Sad, Serbia, using natural infection, and in 1996-7 the remaining 607 of the available PIs were tested in northwestern Minnesota, all in replicated trials (Gulya 1997, 1998; and http://www.ars-grin.gov/cgi-bin/npgs/html/desc.pl?79090). All trials consisted of four replications of single row plots, with a total of ~ 100 plants evaluated. Check varieties included the USDA hybrid 894 and European hybrids known to have Phomopsis resistance at that time (Santa Fe, Albena). Data were recorded at least twice, starting ~ 4 wk after anthesis. Plants were recorded as falling into three categories: 1: with any *Phomopsis* lesions, 2: lodged due to *Phomopsis*, and 3: dead due to *Phomopsis.* Those entries having > 20% on average of *Phomopsis*-affected plants in the first rating were excluded from a second rating, taken ~ 2 wk after the first rating.

USDA- Sclerotinia Stalk Rot. Resistance evaluation data in the USDA-NPGS GRIN system for Sclerotinia has been generated since the 1980s (Gulya, 1981, 1985a), and relied both on natural infection and artificial inoculations. From 1980 until 1996, commercial sunflower fields naturally infected with *Sclerotinia sclerotiorum* were used to evaluate stalk rot, with a standard protocol of four replications of single row plots (6 m long, ~ 25 plants/row). A widely grown public hybrid, such as '894' (HA89 x RHA 274), was included as a long term check. Disease severity was expressed as the percent of plants showing wilting and/or a basal stem lesion at harvest. A series of eight locations in North Dakota and Minnesota were used over a twenty year period, with each abandoned due to variable disease distribution or disease decline to due natural buildup of *Sclerotinia* mycoparasites such as *Coniothryium* and *Sporidesmium* (Gulya et al., 1992; Huang & Kozub, 1991). More recently, we have employed an artificial inoculation method, consisting of *Sclerotinia* mycelium grown on millet, which is deposited in a furrow beside each row a month after planting, at a rate of 80 g/ 6 m row (Gulya , 2004), and this method has been mechanized with a tractor mounted applicator (Gulya et al., 2008) facilitating the inoculation of thousands of rows. Although Sclerotinia head rot will not be discussed in this paper, for artificial inoculation we rely on laboratory produced ascospores which are sprayed onto heads at 25% bloom, and then the entire nursery is misted by an automated system to insure constant wetness, both during the initial inoculation period, and for a duration of at least five weeks post inoculation (Van Decelaere and Miller, 2001; Henson et al., 2001).

VIR - Sclerotinia. Head rot and stalk rot ratings on VIR accessions were made in central Russia at Yekaterinino Experiment Station of VIR, Tambov province, during the period of 1983 to 1998. The experimental field was infested by placing sclerotia into the soil in the autumn, followed by two sprinkler irrigations the following year (at planting and at the onset of bloom). Three-row, non-replicated plots were used, with ~ 42 plants/plot. Disease incidence, done when the reference cultivar Trudovik reaching 100%, was calculated as the percentage of injured plants versus their total number per plot. Large numbers of breeding materials and part of the VIR collection were tested, and this information may be requested from the third author.

RESULTS

VIR - Phomopsis. During five years (2000, 2002, 2006, 2008, 2009) in the last decade the incidence of *Phomopsis* stem canker was high enough at the Kuban VIR Station to warrant taking disease incidence notes, and 1860 accessions were rated in those years. Pooling the data across those five years illustrates the susceptibility of most sunflower accessions (Fig. 1), with 22% of the accessions having 100% plants infected. At the other end of the spectrum, 49 accessions (2.6% of the 1860 tested) were immune with no apparent *Phomopsis* infection, based on one year's data from a 3-row plot for each accession. The *Phomopsis*-resistant accessions originated from 12 different countries, implying there is a substantial

amount of geographic and possible genetic diversity within this group. The largest number of resistant accessions originated from Russian accessions. The accessions which showed no *Phomopsis* infection in the seed increase plots during this time period included the following entries (VIR #, country of origin, pedigree or cultivar name):

a) nine open pollinated oilseed cultivars: #552 (Russia: Zelenka),#1232 (Russia: Omsky Rianniy), #1716 (Russia: Kamyshenskiy); #1959 (Russia: Chkalovskiy Gigant); #1970 (Moldavia,), #2219 (Armenia), #2228 (Russia: Saratvskiy-2); #2229 (Russia: Rannespely); #2232 (Russia: Aurora); #3430 (Russia: Ataman), and #3431 (Russia: Azovskiy),

b) five large-seeded confection type cultivars: #3526 (Russia: Lakomka); #3510 (Russia Donskoi), #3516 (Ukraine: Zaporozhskiy) #3579 (Ukraine: Mestnyi-3), and #3581 (Ukraine: Mestnyi-5),

c) ten populations: #1702 (USA), #1869 (Russia), #1883 (France), #1886 (Romania), #1957 (France), #2037 (Argentina, Girasol la Prevision 9), #2678 (Ukraine), #2688 (Ukraine), #2865 (Armenia), #2875 (Kazakhastan).

d) and 25 oilseed lines: #1961 (Russia, V-8883 selection), #2227 (U.S., HA61-1), #2303 (Canada, CM 198), #2305 (Canada, CM 214), #2310 (Russia, VIR C-17), #2336 (Poland, L-1585U), #2397 (Argentina, 938-39-3-1-3-1-2) #2701 (Ukraine, TA-6463), #2709 (Ukraine, TA-4181-8), #2776 (Russia, VIR 136), #2791 (Russia, VIR 170), #2988 (Bulgaria, SL 2188), #3326 (Russia, VIR 365), #3366 (Mexico, E-6 Andro), #3424 (Russia, VIR 658), #3469 (Russia, VIR 249), #3487 (Russia, VIR 448), #3527 (Russia, VIR 449Rf), #3570 (Russia, VIR 114 x *H. giganteus*), #3571 (France, LR1 #227), #3595 (Russia, VIR 130B), #3614 (France, L440), #3615 (France, L270), #3616 (France, L342), and #3617 (France, L273).

Several of these resistant accessions were also tested by VNIIMK personnel using artificial inoculations, with both mycelia and ascospores, in 2009 in Krasnodar. The cultivar 'Zelenka' (VIR #552) was immune to both natural and artificial infection, and the lines VIR 249, VIR 365, VIR 448 and VIR 449 were rated as highly resistant in these inoculated trials.

USDA - Phomopsis. A third (499 accessions) of the USDA cultivated sunflower collection was evaluated in 1989 in then Novi Sad, Yugoslavia, and the remaining 607 accessions were tested under natural infection in northwestern Minnesota (USA) in 1997 and 1998. The frequency distribution of *Phomopsis* resistance is shown in Fig. 2, which illustrates the low occurrence of resistance. The incidence of *Phomopsis* in the 1989 Novi Sad trial averaged 31%, and accessions ranged from 3 to 74% infected. In the two U.S. trials, the 1996 test averaged 43% infection (range 0 to 100%) and the 1997 test averaged 40% infection (range 3 to 78%) at the first rating. Instead of taking further notes on all entries, the second rating was made only on those accessions which had < 20% infected plants at the first rating. Of the 89 entries rated a second time, there were 23 accessions with < 10% infection (Table 1), which was 2% of the 1106 entries tested in 1996/1997, and one entry (PI 650530) showing no infection. The frequency of *Phomopsis* resistance in the USDA and VIR sunflower collections is thus similar, as illustrated in the two histograms (Fig. 1 and 2). The highly resistant entries in the USDA collection originated from 15 countries, and were diverse in many morphological traits (Table 1). The four traits listed (oil percentage, stem height, days to flowering, and 100 seed weight) illustrate the range of characteristics associated with the highly resistant entries.

USDA - Sclerotina stalk rot. Data from five years of field trials with natural infection of Sclerotinia stalk rot are listed in GRIN (http://www.ars-grin.gov/cgi-bin/npgs/html/desc.pl?79081), for a total of 977 accessions evaluated (after duplicate entries are eliminated). Since this data was generated in years with very different weather and disease incidence, one way of standardizing the data is to express the infection level relative to a check variety, which in this case was public hybrid '894' (USDA lines HA89 X RHA274). Stalk rot incidence on the '894' check for the five trials was 19, 82, 51, 84 and 61%, illustrating the variability of disease pressure. Disease incidence on the entries in the five trials ranged from 0-45%, 36-100%, 11-91%, 23-100%, and 29-100%. Roughly 61% of the accessions had stalk rot higher (100 to 237%) than that of the check, and 39% had less infection (Fig. 3). Nineteen entries (7% of the total) in these replicated trials had infection levels at 25% or less compared to the check variety, and one entry (PI 431560) had no apparent stalk rot infection. The agronomic data, also available in GRIN and listed in Table 2, again demonstrates the morphological and phenological diversity, with the germplasm originated from five countries. Comparing the USDA accessions with *Phomopsis* resistance (Table 1) and *Sclerotinia* resistance (Table 2), it is noteworthy that three entries had high levels of resistance to both pathogens (PI 377530- Kenya, PI 431514- Romania, and PI 431563- Serbia).

As more cultivated sunflower accessions became available from the USDA collection, a second effort was made to evaluate for Sclerotinia stalk rot infection starting in 2007. A total of 250 cultivated PIs were evaluated, but in these tests artificial inoculation with millet-based mycelium (Gulya et al., 2008;)

was used rather than natural infection, and the entries were compared with 12 USDA inbreds developed specifically for Sclerotinia resistance. The tests were conducted in 2008 and 2009 at two locations each year, with two replications, for a total eight, single row plots/ accession, or about 200 plants observed. The 262 entries averaged 41% Sclerotinia stalk rot over the four locations, ranging from 3.5% infection to 84% (Fig. 4). Two commercial hybrids were included for comparison (susceptible Mycogen 270, resistant Croplan 305), and their respective disease severities, averaged over four locations were 39% and 22%. Thus, 30 of the 262 entries (11%) were more resistant than the most resistant commercial hybrid used for comparison. The most resistant entry was USDA line HA 441, which, while developed for head rot resistance, also has high levels of stalk rot resistance. The top 25 entries in this two-year trial are listed in Table 3, along with agronomic characteristics. Within this group of 25 highly resistant entries were five USDA lines HA 377, RHA 409, HA 412, HA 441 and RHA 468 specifically developed for Sclerotinia resistance (Miller, 1992; Miller & Gulya, 1999, 2006; Hulke et al., 2010) which displays the progress made in transferring disease resistance into high oil germplasm combined with other agronomic traits. The remaining 18 highly resistant entries in this two-year trial originated from breeding programs in 12 countries in Africa, Europe, Central and South America, illustrating the wide geographic and presumable genetic diversity. Several of the resistant entries were long-seeded types suitable for inclusion in confection breeding programs. Also noteworthy are germplasm with resistance to multiple disease, illustrated by the Russian cultivar 'Zelenka', with resistance to *Sclerotinia* and *Phomopsis*, and the old Argentine cultivar 'Charata', with both Sclerotinia and rust resistance (developed into USDA line HAR-4; Gulya, 1985b).

DISCUSSION

The preceding results highlight the diversity of cultivated sunflower maintained by two large public germplasm collections of cultivated sunflower, and some of the disease evaluations performed on them. Data on the USDA-NPGS sunflower collection, with regard to disease, insect and agronomic traits, is all available online, allowing researchers to quickly identify sources of resistance. Since seed is freely available, with minor importation restrictions, this germplasm collection represents a readily available source of genes for disease resistance. The VIR collection is another excellent starting point for disease resistance genes. Since the geographic composition of the VIR collection is also large, but concentrates on material originating from Russia and the former Soviet Union, the genetic background of the VIR and USDA collection are different and compliment each other. Disease evaluations of the VIR collection, primarily for *Phomopsis* and *Sclerotinia* resistance, have been made relying upon natural infection, and while not available online, they can be requested from the third author.

The various USDA and VIR disease evaluations illustrate some of pitfalls associated with generating disease resistance ratings. Relying upon natural infection, there is a significant yearly variation in disease intensity which makes comparisons between entries from different trials a statistical challenge. One method to allow such comparison is to standardize the data relative to a common check variety. Another experimental approach to minimize annual variation is to use artificial inoculations which should not only increase disease severity, but also minimize experimental variation. Environmental interaction with host and pathogen, however, will never be eliminated. Thus, testing across multiple environments, at least of the most resistant material, will generate data representing the true disease resistance potential of any sunflower germplasm.

Due to the scope of this paper, no effort was made to go into detail on resistance to other pathogens. However, the USDA cultivated sunflower germplasm collection has been evaluated for other diseases as mentioned in the Introduction. These diseases include multiple races of rust (*Puccinia helianthi*), multiple races of downy mildew (*Plasmopara halstedii*), white rust (*Albugo tragopogonis*), and Verticillium wilt (*Verticillium dahliae*). Several other diseases do not occur in the U.S. with enough severity to allow tests with natural infection or they are not economically serious at present to warrant efforts with artificial inoculation. Thus, for diseases like Alternaria blight, Septoria blight, powdery mildew, charcoal rot (*Macrophomina phaseolina*), and Rhizopus head rot, there is currently no resistance data available for USDA germplasm. Finally, for some diseases the best sources of resistance are wild *Helianthus* species, and thus USDA research efforts on some diseases, such as *Septoria* and *Alternaria* leaf blights, have focused on wild species rather than cultivated sunflower.

The value of the Russian and U.S. germplasm collections is highlighted in the number of publically released lines developed specifically for disease resistance. Within the USDA Sunflower unit, where the recent focus has been on Sclerotinia, with lesser emphasis on rust and downy mildew, numerous lines have been developed using cultivated germplasm from the USDA collection and in other cases from commercial hybrids. For example, Sclerotinia stalk rot resistance and rust resistance was obtained from

USDA PIs (Miller and Gulya, 1999 and 2001) while head rot resistance in other releases was derived from commercial hybrids (Miler and Gulya, 2006). Resistance to a new strain of Verticillium was found in one USDA PI within the core collection (Radi and Gulya, 2007), and resistance to *Albugo,* screened under natural epiphytotics in South Africa, was found in eight of 1168 accessions (Gulya et al., 2000). Resistance to *Orobanche* was identified in cultivated germplasm (Gulya et al., 1994) to some of the 'early', less virulent races, but resistance to newer races has primarily been found in wild *Helianthus* (Fernández-Martínez et al. 2008). Many of the USDA germplasm releases have complicated pedigree (partially mapped by Korell et al., 1992), with disease resistance often coming from US and Russian material, interspersed with genes derived from wild sources. A similar pedigree map has been made for publically released Argentine cultivars, many of which derive disease resistance from older Russian germplasm (Romano and Vazquez, 2003).

In summary, resistance to many important sunflower pathogens has been found in cultivated sunflower accessions maintained in public germplasm collections. Since the germplasm within most public collections is freely distributed to public and private plant breeders, these collections represent an immediate, diverse source of potential resistance donors. As information on disease resistance is generated and shared with the germplasm curators, the challenge for individual plant breeders to obtain sources of resistance is lessened. In some cases, however, disease resistance to certain pathogens is extremely rare within cultivated germplasm, and the sunflower research community is fortunate to have access to a wide diversity of wild *Helianthus* species. While some of the polyploid, perennial *Helianthus* species present more of a challenge to transfer genes to diploid cultivated sunflower, the extensive pool of wild germplasm (much already in public collections) is a virtual goldmine of genes for disease resistance and other traits. Thus, between cultivated sunflower and wild *Helianthus* species, plant breeders have a wealth of genetic resources readily available to them in their quest to find new sources of disease resistance.

PI #	*Phomopsis* %	Pedigree/Variety Name	Origin	Oil (%)	Ht (cm)	Days to Flwr	100 sdwt (g)
650530	0	371-3 S	Canada	42	105	60	6.4
162784	3	No. 167	Argentina	29	245	74	8
650657	3	Ames 10101	China	28	240	80	15.1
219649	3	Lodging Resistant	Austria	26	210	70	6.2
650701	4	CO-PB 88	Spain	30	430	127	5.9
162454	5	Sunrise	Uruguay	27	165	67	5.8
431563	5	R-201/4	Serbia	41	210	65	8
650754	6	HA-R3	U.S.A.	34	210	72	5
377530	6	Kenya White	Kenya	32	265	83	8.1
431562	6	PO 30/3-1	Serbia	37	120	62	6.3
650659	7	Ames 10103	China	36	195	75	14.1
170393	7	No. 2008	Turkey	25	255	81	5.3
170385	8	No. 1397	Turkey	26	255	72	6.7
170426	8	No. 3447	Turkey	30	240	64	8.3
171656	8	No. 6874	Turkey	26	300	81	9.7
174217	8	No. 8149	Turkey	27	270	101	6.5
181769	8	Tournesol	Lebanon	27	335	87	7.6
250085	8	Abadsens	Egypt	26	270	58	5.9
650413	8	Kruglik A-41	Germany	33	105	61	7.6
507896	8	3100399	Hungary	24	185	74	10.2
431567	9	VNIIMK 8883 4/1-1	Serbia	37	135	74	4.2
433862	9	Giza	Serbia	31	320	58-91	14.2
494857	9	ZFA 3225	Zambia	32	*ND*	*ND*	9.2

Table 1. Description of USDA cultivated sunflower Plant Introductions with highest levels of *Phomopsis* resistance (of 1106 PIs tested in replicated trials at three locations under natural infection). Full dataset can be found at http://www.ars-grin.gov/cgi-bin/npgs/html/desc.pl?79090).

PI#	Stalk Rot (% of Check)	Stalk Rot (actual %)	Origin	Pedigree/Varity Name	Oil	Ht (cm)	Days to Flwr	100 Sd/Wt
431560	0	0	Serbia	PO 64/2	23	145	68	4.3
377530	5	1	Kenya	Kenya White	32	265	83	8.1
431502	5	1	Poland	PL 7937-175	36	100-120	73	6.8
431558	5	1	Serbia	NS-B 16-63/1	42	150	66	4.3
431563	5	1	Serbia	R 201/4	41	210	66	8
431514	11	2	Romania	Romsun C-5357	29	105	68	7.3
431568	11	2	Serbia	V 8883 4/2-1	33	145	64	7.1
431521	16	2	Romania	V-1352	29	95	67	6.8
431525	16	3	Russia	VK-6	33	110	61	7.4
431529	16	3	Russia	VI-32	32	125	59	6.8
431541	16	3	Serbia	D-75-8	28	190	77	7.4
431561	16	3	Serbia	PO 19/1-1	35	150	62	6.6
431507	21	4	Poland	T 6556-1-2	37	200	63	6.7
431539	21	4	Serbia	D-75-5	25	160	75	5.5
431544	21	4	Serbia	D-75-12	33	160	70	5.0
431557	21	4	Serbia	NSB-16-61/6	43	160	68	3.4
431569	21	4	Serbia	V 8931 2/2-1	27	125	60	10.7

Table 2. USDA cultivated sunflower Plant Introductions (PIs) showing highest levels of resistance to Sclerotinia stalk rot in multiple years' naturally infested trials (U.S.), with agronomic data available from the GRIN database (http://www.ars-grin.gov/cgi-bin/npgs/html/desc.pl?79081).

P.I.#	Stalk Rot %	Origin	Pedigree	Oil %	Ht (cm)	Days to Flwr	100 Sd/Wt
HA 441	*3.5*	USDA	HA 412/SD	45	160	61	
650778	*6.9*	Russia	Harkouski-101	44	210	68	9.6
600714	*7.8*	Spain	CO-PB 105	28	225	75	9.0
650786	*10.9*	Russia	VNIIMK 3497	47	205	67	6.8
535890	*11.9*	Poland	Krzynowloski Miejscowy	26	225	70	12.6
650613	*12*	France	HIR 34	26	145	67	3.9
650787	*14.1*	Russia	VNIIMK 1696	39	215	69	8.8
531389	*14.4*	Czech	Slovenska Siva	32	140	65	5
480471	*14.5*	Zambia	FS-a-3	39	175	67	5.2
RHA 377	*14.6*	USDA	RHA 299//SOREM HT 58/RHA 801				
531366	*15.3*	Poland	Lengyle - A	32			
650562	*15.3*	USDA	High Oil 74	43			
531359	*15.4*	Germany	HZ-SM 27.208	27			
RHA 409	*15.6*	USDA	Romania R-line SCL Rec Cycle C2				
531361	*15.6*	Hungary	Iregi Hnk 81	37			
600725	*16*	USDA	BRS-3 (bird resistant synthetic)				7.7
650525	*16*	Canada	S8 A9343 2/3-3	39	145	68	6.5
650757	*16.5*	Mexico	E-3 normal				
650810	*17.1*	Paraguay	Guaran				
650676	*17.3*	Spain	CO-PB-42				
RHA 468	*17.6*	USDA	RHA 428/RHA 426//RO 12-13/3/RHA 274/PRS 5				
650831	*18.1*	Russia	Zelenka				
HA 412	*18.5*	USDA	USDA B/SCL B-3 Cycle 1				
650541	*18.5*	Argentina	Charata				
650807	*19.3*	France	Dussol				

Table 3. USDA cultivated sunflower Plant Introductions (PIs) and released inbred lines showing highest levels of Sclerotinia stalk rot resistance in multiple years' artificially inoculated field trials of 263 entries, with agronomic data available from the GRIN database.

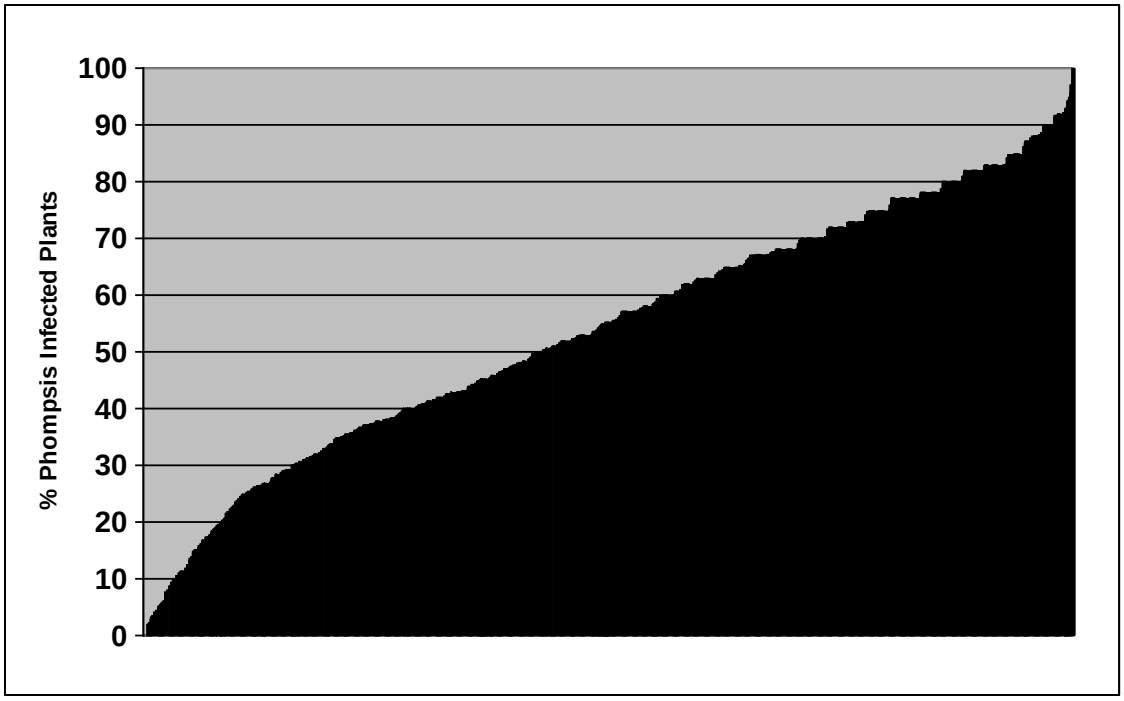

Fig. 1. *Phomopsis* resistance in 1006 USDA cultivated Plant Introductions evaluated in three naturally infected field trials in Novi Sad, Serbia (1989) and Minnesota, U.S. (1996-97). A second disease rating on those accessions with <20% Phomopsis infection failed to find any immune accessions.

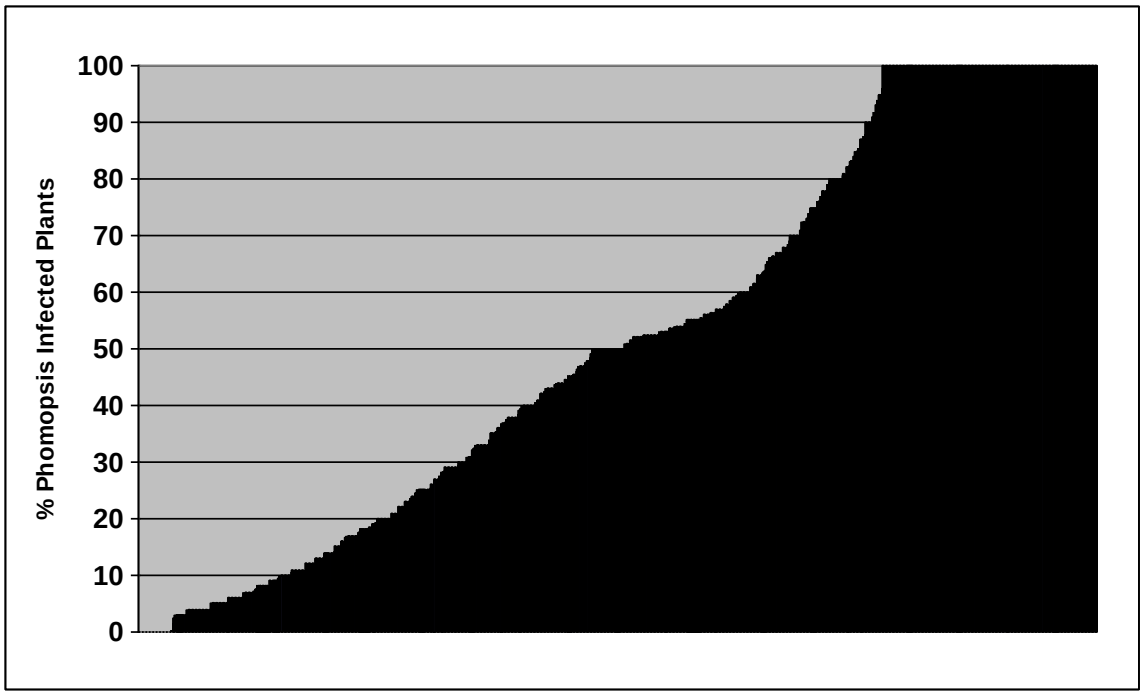

Fig. 2. *Phomopsis* resistance in 1860 VIR cultivated sunflower accessions evaluated over five years (2000, 2002, 2006, 2008, 2009) with natural infection in the Kuban VIR station at Botanika, Russia. Totally susceptible accessions accounted for 22 % of the entries while 45 immune accessions comprised 2.6%.

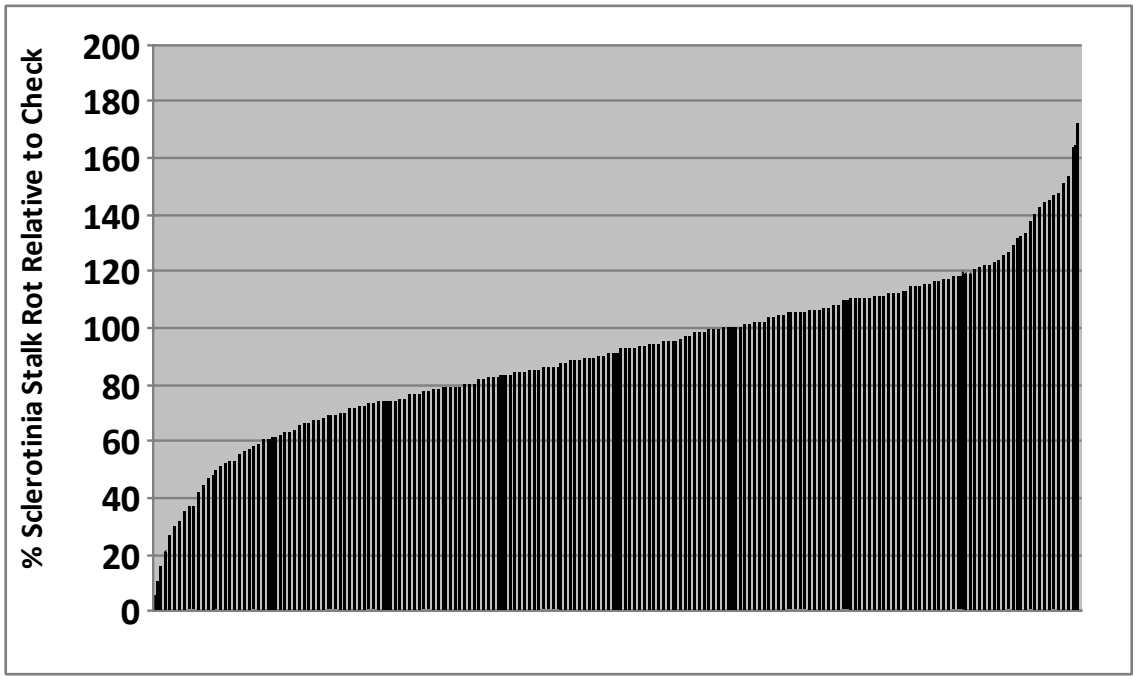

Fig. 3. Sclerotinia stalk rot resistance of 977 USDA cultivated sunflower Plant Introductions tested with natural infection in six U.S. locations, with disease expressed relative to the check variety (USDA hybrid 894 = 100).

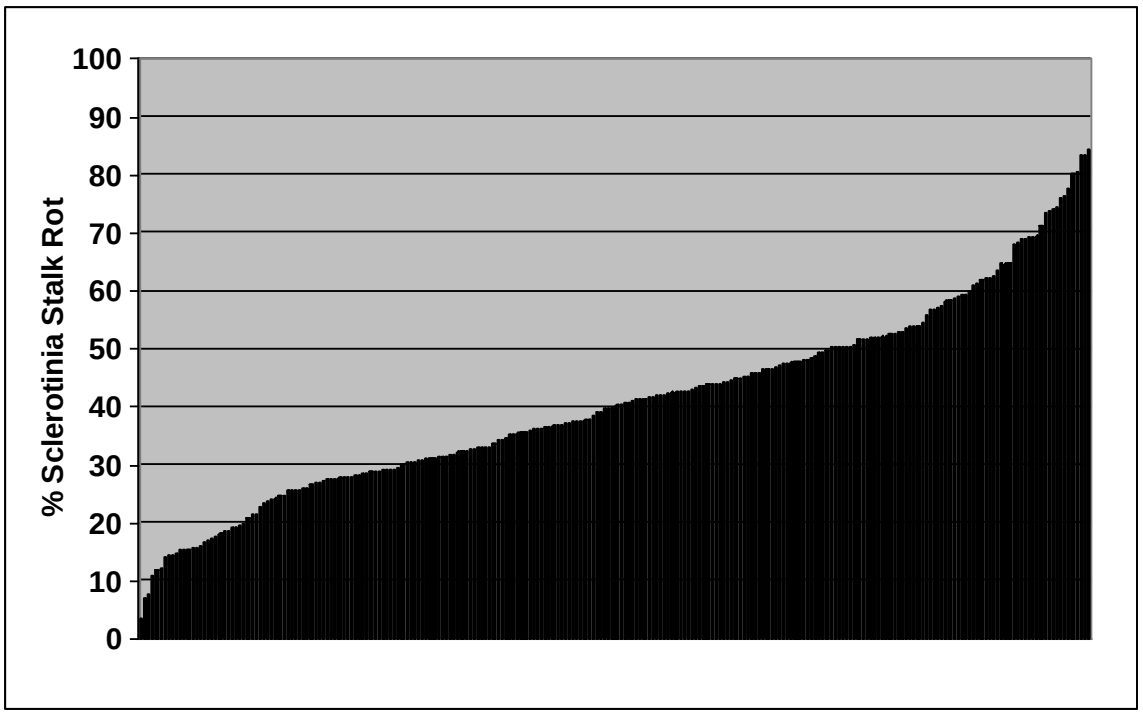

Fig. 4. Sclerotinia stalk rot resistance of 250 USDA cultivated Plant Introductions and 12 released USDA inbred lines, tested with artificial inoculations at four locations over two years.

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Sunflower breeding for resistance to broomrape (*Orobanche cumana* wallr.)

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ABSTRACT

The parasitic angiosperm broomrape (*Orobanche cumana* Wallr) causes economic damage in sunflower production in a number of countries around the world, but especially in Central and Eastern Europe, Spain, Turkey, Israel, Iran, Kazakhstan, and China. For almost a century, there has been a constant tug-of-war between sunflower breeders and *Orobanche cumana*, with frequent changes in which side has the upper hand. Almost as soon as the breeders find a source of resistance to the latest race of the pathogen, broomrape responds by evolving another virulent race. Sunflower selection for broomrape resistance makes use of different methods for testing breeding materials (in the field, greenhouse, or at the molecular level), looks for resistance sources in certain wild species of the genus *Helianthus*, and has so far produced significant results. Dominant genes for resistance to races A, B, C, D, E, and F have been found and incorporated into cultivated sunflower genotypes.

In the last two to three years, however, new broomrape populations have been discovered in several different countries (two each in Romania, Russia and Turkey, one in Spain, and most likely another one in Ukraine). None of the existing commercial hybrids resistant to races A, B, C, D, E, and F have proven resistant to these new populations of the pathogen. Fortunately, greenhouse testing conducted by the Fundulea Institute in Romania in 2009 has managed to identify two restorer lines that are resistant to all the new populations and can be used directly in developing hybrids.

Sunflower breeders and geneticists have achieved significant results in the use of molecular markers for identifying new broomrape races (A-F). Marker-assisted selection should be used even more in the future search for Orobanche resistance.

Broomrape can also be managed by the development of IMI-resistant hybrids or by using biological control measures. In parallel with the search for broomrape resistance genes, efforts should be made to alter the anatomy of plant organs as well as biochemical parameters (mechanical barriers, germination inhibitors, phytoalexins, etc).

To speed up the progress of sunflower breeding for resistance to Orobanche, there should be a greater level of collaboration between the breeders from public institutions and private companies.

Key words: breeding – broomrape – molecular markers – races – resistance – sunflower

INTRODUCTION

Broomrape (*Orobanche cumana* Wallr. = *Orobanche cernua* Loelf.) is a parasitic angiosperm that has been causing a great deal of damage to sunflower production for more than a century. According to Morozov (1947), the first reports of broomrape in sunflower came from Saratov Oblast in Russia and date back to the 1890s. The same author mentions that the first sunflower varieties resistant to race A of *Orobanche* were developed by Plachek (1918) at the Saratov breeding station. Morozov (1947) and Pustovoit (1966) both note that Ždanov (1926) identified a new broomrape race (B) in Rostov Oblast and soon after the discovery developed a number of sunflower varieties resistant to it. In the period that followed, according to Pustovoit (1966), a number of high-oil varieties resistant to race B were developed at the VNIIMK institute in Krasnodar, Russia that thereafter played an important role in the spread of sunflower around the world. Later on, a new race that could not be controlled by the genes for resistance to races A and B was discovered in Moldova by Sharova (1968) and in Bulgaria by Petrov (1970). Through genetic research, Vranceanu et al. (1980) established that there were five broomrape races in Romania and identified dominant genes controlling resistance to them. Alonso et al. (1996) found a new, virulent race (F) of the pathogen in 1996 in Spain. Papers by Alonso et al. (1996), Škorić and Jocić (2005), and Fernandez-Martinez et al. (2007) each provide a detailed overview of the achievements of sunflower breeding for resistance to *Orobanche*.

Extensive research on broomrape resistance has been conducted in countries of the former USSR as well as in Romania, Bulgaria, Turkey, and Spain. In all these countries, broomrape causes great damage to sunflower production and new races of the pathogen appear frequently. In addition to Russia, Ukraine, Romania, Bulgaria, Turkey, and Spain, broomrape is also present in Serbia, Hungary, Moldova, Greece, Israel, Iran, Kazakhstan, China, Mongolia, and Australia, and possibly in a few other countries as well. Sunflower breeders and geneticists have been trying to develop genotypes resistant to all known races of the parasite.

The objective of this paper was to make an overview of what has been achieved in sunflower breeding for *Orobanche* resistance so far and to describe the sources and genetics of this resistance as well as the breeding methods and directions employed in the field.

SOURCES OF BROOMRAPE RESISTANCE

Genes for resistance to broomrape races A, B, C, and D are present in varietal populations of sunflower developed in breeding programs from Krasnodar, Armavir, Odessa, Fundulea and several other places. Genes that confer resistance to races E, F, G and the latest ones, on the other hand, have been identified in certain wild species of the genus *Helianthus* and have been incorporated into cultivated sunflower genotypes by interspecific hybridization. A species of wild sunflower (*Helianthus tuberosus*) was first used as a source of *Orobanche* resistance by Ždanov in the 1930s (Morozov, 1947). Later on, Galina Pustovoit (1975) and her team made a great contribution in this area by developing sunflower varieties through interspecific hybridization in which *H. tuberosus* was used as the donor of *Or* genes. These varieties were used in the identification of Or_5 and Or_6 genes. Confirmation of this can be found in a study by Venkov and Shindrova (2000), who over a 10-year period tested six of Galina Pustovoit's sunflower cultivars for resistance to broomrape races present in different parts of Bulgaria. The study's findings showed that the Russian varieties Progress and Oktobar, the Bulgarian variety Vega, and the Romanian hybrid Sorem 80 all had stable resistance to the latest races of *Orobanche* (D + E) found in Bulgaria at the time.

In more recent times, a number of authors have used wild *Helianthus* species in their search for resistance to broomrape. Thus, Ruso et al. (1996) tested wild sunflower species for resistance to three virulent races of *Orobanche* and determined that most of the perennial species examined were immune to the three races. Furthermore, some of the annual wild species and lines obtained by interspecific hybridization were resistant as well. Sukno et al. (1998) crossed several wild species (*H. resinosus*, *H. pauciflorus*, *H. laevigatus*, *H. nuttallii* ssp. *nuttallii*, *H. giganteus*, etc.) of sunflower with cultivated sunflower genotypes and obtained plants of the F_1 and BC_1F_1 generation as well as some BC_2F_1s. The wild species and interspecific hybrids all proved resistant to broomrape infestation except for the species *H. nuttallii*, in which segregation occurred, indicating that the resistance was dominant.

Jan et al. (2000) describes the procedure for and the results of transferring genes for resistance to broomrape from perennial wild sunflower species into cultivated sunflower genotypes. Three Spanish populations of *Orobanche cumana* Wallr. were involved. The results of the study showed that two of them could be controlled by the Or_5 gene, while the third was the virulent race F.

Fernandez–Martinez et al. (2000) tested 54 wild sunflower accessions (representing 27 perennial and four annual species) and 55 cultivated sunflower accessions, which they raised in a growth chamber and then transplanted to a greenhouse. The material was inoculated with the virulent race F (population SE 296). Most of the perennial species proved fully resistant to race F. The only exceptions were some populations of four of the wild perennials, which had a certain percentage of susceptible plants. Among the wild annual species, *H. anomalus* and *H. agrestis* were completely resistant, while *H. debilis* ssp. *cucumerifolius* and *H. exilis* segregated with regard to *Orobanche* resistance.

Jan and Fernandez-Martinez (2002) employed interspecific hybridization to incorporate genes for resistance to race F from several wild species into cultivated sunflower. Where necessary, they used embryo culture and chromosomal doubling by colchicine in order to bypass the barriers and enable the transfer of desirable genes. The newly developed genotypes had resistance to race F, which was controlled by a single dominant gene.

An overview of how races of broomrape progressed from race A to F in Spain over time can be found in Melero-Vara et al. (2000). Cultivated sunflower genotypes were found to have a low frequency of genes controlling races E and F, while of the 18 annual species studied, only *H. agrostis* and *H. anomalus* exhibited full resistance. Among the wild perennials involved in the study, 74% of the species were fully resistant to races E and F, while 11% showed segregation concerning resistance to race F. Genetic

analysis showed that resistance to races A through E is by and large controlled by a single dominant gene. In some cases, two dominant genes, epistatic interaction, and reversal in the dominance were observed.

Jan et al. (2002) crossed the wild sunflower species *H. maximilianii* Schrad, *H. grosseserratus* Mart., and *H. divaricatus* L. with cultivated sunflower and developed four populations (BR1-BR4) resistant to race F in Spain.

According to Fernandez-Martinez et al. (2007), research on the resistance of sunflower germplasm to different broomrape races has shown that wild *Helianthus* species are the main source of resistance to the new, virulent races of the pathogen. Still, cultivated sunflower genotypes, especially those developed by interspecific hybridization, cannot be completely disregarded as a source of genes for broomrape resistance. The use of molecular markers should also provide further clarification of the genetic control of broomrape resistance in sunflower.

Christov et al. (1992, 1998, 2009) have achieved outstanding results in identifying genes for broomrape resistance in the wild species of the genus *Helianthus* and incorporating them into cultivated sunflower genotypes. Especially important are the findings reported in Christov et al. (2009), which concern the detection of *Or* genes in 11 perennial wild sunflower species and their incorporation into elite cultivated sunflower lines by means of interspecific hybridization.

Sources of *Orobanche* resistance can also be found by the use of induced mutations. Venkov and Shindrova (1998) reported that they obtained a mutant with partial resistance to *Orobanche cumana* Wallr. using a 0.4% solution of the mutagen nitrosomethylurea.

METHODS USED FOR EVALUATING BROOMRAPE RESISTANCE IN BREEDING PROGRAMS

In order to attain their breeding goals and identify sources of broomrape resistance, sunflower breeders must develop a breeding strategy, decide on a breeding method, secure the necessary germplasm and differential lines for broomrape race identification, and choose the appropriate inoculation method and molecular marker technique (MAS). To ensure the success of the program, the best way to go is to pick out an elite line and cross it with a source of *Or* genes, which should then be incorporated into the breeding material using certain techniques in order to create new genetic variability. At the start of the program, the breeder must determine which race or races are present in the region for which the hybrids are being developed. A set of differential lines for races A, B, C, D, and E has been provided by Vranceanu et al. (1980), while Pacureanu-Joita et al. (1998) have identified such a line for race F. As of yet, there are no differential lines for the new, virulent races of this pathogen that have appeared in the last few years. Developing a set of such lines would be desirable.

In the years in which races A through E were discovered, sunflower breeders tested their breeding materials in the field, usually on plots that had been severely infested by broomrape the year before. This method is still employed by some breeders. However, this approach does not always produce reliable results due to the influence of environmental factors and an inadequate amount of broomrape seeds in the soil. In an effort to avoid this, breeders resorted to collecting broomrape seeds and incorporating them into the hills in which sunflower seeds were placed at planting. This method too, however, is prone to producing experimental errors, caused primarily by the effects of environmental factors. Much more accurate results can be obtained by putting broomrape seeds into containers filled with a pre-prepared medium (soil + some other substances), which are then placed in the controlled environment of a growth chamber or greenhouse. Panchenko (1975) developed a screening method for assessing resistance to broomrape in greenhouse conditions during autumn and winter. This method was further honed by Grezes-Besset (Rustica Prograin Genetique), who made testing using plastic test tubes part of the procedure. The advantage of this technique is that it provides a higher level of reliability and makes it possible to test a large number of genotypes in a short period of time. Labrousse et al. (2004) has recently developed a new method based on hydroponic co-culture, which has been producing outstanding results. However, the most reliable and most easily applicable method of screening breeding materials for broomrape resistance is the use of molecular markers. QTL, RFLP, RAPD, TRAOP, and SSR markers have so far been used for this purpose.

GENETICS OF SUNFLOWER RESISTANCE TO *OROBANCHE CUMANA* WALLR.

In parallel with the appearance of new broomrape races and sources of broomrape resistance, the genetics of resistance to this pathogen has been studied as well. As sources of resistance to races A and B were identified by Plachek (1918) and Ždanov (1930), respectively, it was also determined that resistance

to the pathogen was controlled by dominant genes. Burlov and Kostyuk (1976) and Pogorletsky and Geshele (1976) studied the genetic basis of *Orobanche* resistance and discovered that it was controlled by a single dominant gene, which they labeled *Or*.

Vranceanu et al. (1980) conducted extensive genetic research as part of his study of broomrape in Romania from 1976 to 1980. They established that there were five pathogenic races of this parasite and labeled them A, B, C, D, and E. They also identified a set of differential lines that had cumulative resistance to the five successive races, conferred by the dominant genes Or_1, Or_2, Or_3, Or_4, and Or_5, respectively. When race F subsequently appeared in Romania and resistance to it was discovered in the line LC-1093 (Or_6) by Pacureanu-Joita et al. (1998), this cycle of genetic research was completed.

The appearance of new broomrape races in Spain triggered a new cycle of large-scale genetic analyses. Dominquez et al. (1996) noted that there is a low frequency of genes for resistance to race E in cultivated sunflower and that this resistance is controlled by two dominant genes.

Sukno et al. (1999) reported that resistance to race E is controlled by a single dominant gene. They tested sunflower lines for resistance to broomrape populations from different regions and found that only two were fully resistant to the pathogen. They assumed that the resistance was conferred by additional dominant alleles at the *Or* locus or by a cluster of very tightly linked non-allelic genes. The two lines were shown to be resistant to the new *Orobanche* populations that are able to overcome the Or_5 resistance gene. Alonso (1998) noted that, the known dominant genes notwithstanding, resistance to *Orobanche* may be more complex than previously thought and that genes other than single dominant ones may also be involved. In some cases involving cultivated sunflower germplasm, resistance to race F is controlled by recessive genes. Thus, *Orobanche* resistance found in the lines P-96 and KI-534 is controlled by recessive alleles at two loci (Rodrigez-Ojeda et al., 2001; Akhtouch et al., 2002). The same recessive genes control resistance to race E in the line KI-534 (Rodrigez-Ojeda et al., 2001). Akhtouch et al. (2002) crossed lines resistant to race F with those that are susceptible to it and found segregation ratios of 1:15 and 1:3 in the F_2, F_3, and BC_1 generations, which in most cases indicates double dominant epistasis. Cases of segregation ratios of 3:13 and 1:1 were also recorded in the F_2s and BC_1s, which is indicative of dominant-recessive epistasis. Perez-Vich et al. (2002) tested some interspecific hybrids (cultivated sunflower x *H. divaricatus* and *grosserratus*) in combination with a susceptible genotype and found that the resultant lines had a single dominant gene for resistance to race F in the segregating generations (F_2 and BC_1F_1).

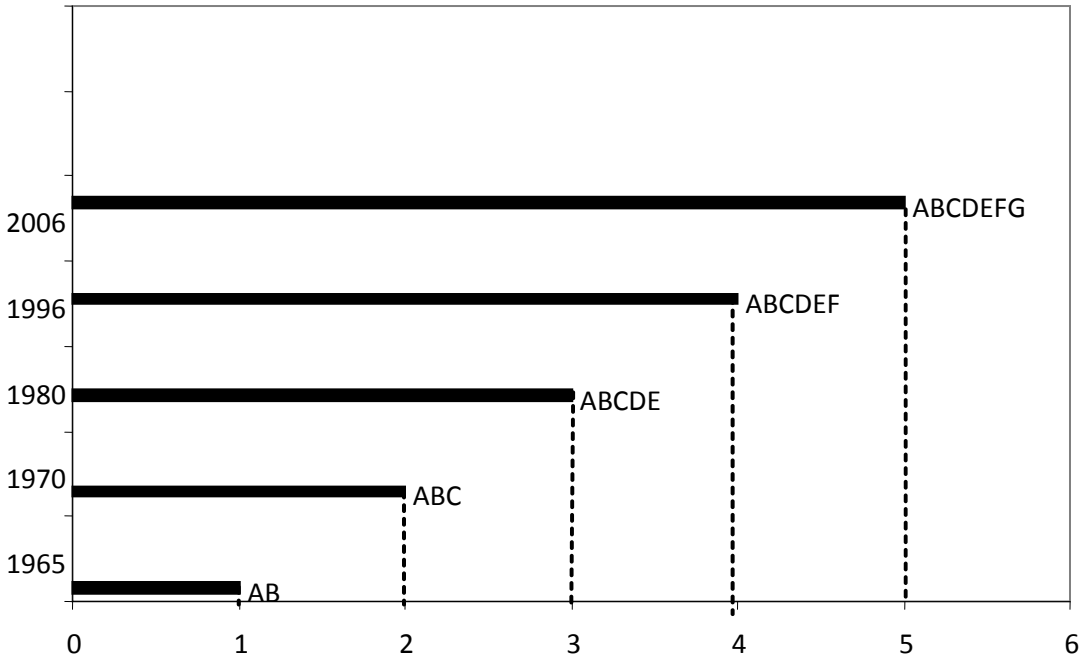

Fig. 1. The evolution of the broomrape races in sunflower crop in Romania

Velasco et al. (2007) crossed a line resistant to race F (JI) with three susceptible lines and obtained segregation ratios of 3:1, 13:3, and 15:1 (R + MR +S) in the F_2, F_3, and BC_1 generations, indicating incomplete dominance of the Or_6 alleles and the presence of a second gene, Or_7, whose expression was influenced by the environment.

Changes in the race composition of broomrape in Romania have been reviewed by Pacureanu-Joita et al. (2008). The findings of the study show that every ten years or so at least one new race of *Orobanche* has appeared in that country, and sometimes even more. Using differential lines for races E and F, Pacureanu-Joita et al. (2004) determined that there are certain differences between the broomrape races found in Turkey and populations appearing in the Constanta region in Romania. The same author also found that *Orobanche* populations from Serbia and the Calarasi county in Romania differ completely from broomrape races present in Spain, some parts of Romania, and Turkey.

Pacureanu-Joita et al. (2008) tested the latest, virulent race of broomrape from Romania through a cross between the resistant line AO-548 and the susceptible line AD-66 and their F_2 and BC_1F_1 generations, in which segregation ratios of 15:1 and 3:1 were observed, indicating that the resistance of AO-548 to the latest race of this pathogen is controlled by two independent dominant genes.

The latest research conducted in Romania under field and greenhouse conditions has shown that sources of resistance to the newest populations of *Orobanche* found in Romania, Turkey, and Spain are present in the lines LC-009 and AO-548 (Table 1). The genetics of this resistance and the race composition of the new populations remain to be studied.

Table 1. Infestation with ten populations of broomrape (*Orobanche cumana* Wallr.) for four sunflower genotypes (Fundulea, 2008-2009)

Orob. pop. / Sun. Gen.	Romania				Russia				Turkey	Spain
	Tulcea	Brăila	Constanța	Călărași	Krasnodar	Stavropol	Rostov 1	Rostov 2		
LC 1093	5/9	0/10	3/10	0/9	0/7	2/10	6/10	7/10	4/10	3/9
	4/10	0/8	4/9	0/10	0/9	0/10	5/10	4/8	5/10	5/10
	7/10	0/9	4/10	0/7	0/8	1/9	5/9	6/10	3/9	4/10
	5/10	0/10	3/10	0/10	0/10	1/7	7/9	7/10	4/8	3/8
	5/9	0/10	3/9	0/9	0/6	2/10	6/10	6/10	5/10	4/8
LC 009	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
PR64A71	1/10	0	0	0	0	0	1/9	1/10	2/10	1/9
	0/10	0	2/10	0	0	0	0/9	0/10	6/9	0/8
	2/10	0	0	0	0	0	0/10	1/10	1/10	1/10
	0/9	0	1/9	0	0	0	2/10	0/9	0/10	2/10
	2/10	0	0	0	0	0	0/10	0/10	1/8	0/9
LC 009	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0

In the Trakya region in Turkey, the race composition of the broomrape populations changes frequently. According to Bulbul et al. (1991), race E was dominant from 1983 to 1990, after which race F appeared. Recently, as reported by Kaya et al. (2004), at least one new race of broomrape has appeared in the country that cannot be controlled by the Or_6. Kaya et al. (2009) have reported that genes for resistance to the newest, virulent race of *Orobanche* have been found in several lines and hybrids.

Races A and B were dominant in Bulgaria until 1968. Then, Petrov (1970) reported that a new race of the pathogen had appeared that could not be controlled by the Or_1 and Or_2 genes. Shindrova (2006) made an overview of the broomrape races found in Bulgaria. According to her findings, races D, E, and F are present in the country. Race E is the most widely distributed one, race F is spreading, while race D is disappearing.

Studies by Antonova et al. (2009) and Goncharov (2009) both discuss the dynamic change of broomrape races in Russia. It is known that *Orobanche* races change frequently in Ukraine and Moldova too, and that, although no public reports have been made yet, there are at least seven races of the pathogen in the two countries. According to Škorić and Jocić (2005), race E is the dominant form of broomrape in Serbia. *Orobanche* has been present in China for a long time too, and identification has been made of race A by Baichun et al. (1996). New races have appeared in the country since, but the race composition has not been determined yet.

Molecular research for the purposes of race characterization and mapping is developing rapidly. Melero-Vera et al. (1996) used RFLP for the characterization of broomrape races. Lu et al. (1999) determined that there is a linkage group that contains the Or_5 gene conferring resistance to Orobanche cumana Wallr race E. These findings confirm that the Or_5 linkage group could be integrated with the linkage group 17 of the GIE Cartisol RFLP map. According to Tang et al. (2003), the Or_5 has been mapped to the end of LG3 distal to the marker loci. Perez-Vich et al. (2004) analyzed resistance of the line P-96 to races E and F at the molecular level. Based on a linked map comprising 103 marker loci distributed on 17 linkage groups, it was determined that only five QTLs (or1.1, or3.1, or7.1, or13.1, and or13.2) were responsible for resistance to race E, while only 6 QTLS (or1.1, or4.1, or5.1, or13.1, or13.2, and or16.1) controlled resistance to race F and were found on seven of the 17 linkage groups. These results suggest that sunflower resistance to broomrape is controlled by a combination of qualitative, race-

specific resistance effecting the presence or absence of broomrape and quantitative, non-race-specific resistance affecting the number of broomrape stalks per plant.

Ioras et al. (2004) used RAPD and SSR markers in the detection of broomrape resistance and determined that the RAPD markers USC 73, UBC 318, UBC 264, UBC 685, and OP-A 17 and the SSR markers ORS 1:14 and ORS 1036 can be successfully used for such detection.

Marquez-Lema et al. (2008) used TRAP and SSR markers responsible for the Or5 gene and were able to map efl-alfa (elongation factor 1-alfa) chit. (chitinase, PR 3 protein) and HaACl (aldo-keto reductase) loci to LGs 7, 9, and 17, respectively, none of which were co-located to Or_5. These results were partially expected, since the chit. and HaACl genes play a role in defense responses and efl-a is a housekeeping gene, and dominant race-specific genes such as Or_5 are hypothesized as essentially playing a role in an early stage of the plant-pathogen interaction.

A study by Joel et al. (2004) confirmed the importance of molecular markers for the study of sunflower resistance to *Orobanche*. They found that RAPD patterns of DNA extracted from soil-borne *Orobanche* seeds is identical to that of DNA from vegetative plant material, provided that the seeds had not deteriorated. They also note that DNA of reasonable diagnostic quality could be extracted not only from tetrazolium-positive soil-borne *Orobanche* seeds but also from tetrazolium-negative seeds. This makes it possible to perform quick genetic analysis without having to wait for broomrape seeds to germinate or develop into plants.

It is very important to know all the mechanisms of broomrape resistance (physiological, biochemical, mechanical, etc.) in order to be able to understand all aspects of this phenomenon. These resistance mechanisms have been studied for a long time. Thus, Morozov (1947) cites the results of Richter (1924) that indicate that broomrape-susceptible sunflowers have root systems with a low pH. The same author also found that broomrape from Saratov Oblast in Russia (race A) had two physiological thresholds – one in acidic soils, up to which broomrape seeds germinate easily, and one in alkaline soils, beyond which susceptible cultivars become "resistant" and no broomrape infestation occurs.

Morozov (1947) also cited the results of Suhorukov (1930) concerning the link between peroxidase values and sunflower susceptibility to broomrape, according to which increased soil acidity increased peroxidase activity and the susceptibility of sunflower plants to *Orobanche*. Much later, Antonova (1978) showed that the action of peroxidases excreted by the parasite was involved in the lignification of host cells.

Antonova and Ter Borg (1996) reported that differences in peroxidase production can be used for interpreting the different virulence of races A and B as well as to explain the gene-for-gene interaction between sunflower and broomrape.

According to Morozov (1947), Barcinskiy (1932, 1935) reported that sunflower root cells contain substances that stimulate the germination and development of broomrape seeds and seedlings. Long after that, Wegmann (1998), Alonso (1998), Matusova et al. (2004), and Honiges et al. (2009) also pointed out the importance of broomrape germination stimulants. The most widely known such stimulants are strigol, electrol, orobanchol, and the synthetic stimulant GP 24.

Matusova et al. (2004) studied germination stimulants as well. Their results indicate that parasitic weed seeds are highly sensitive to the germination stimulant GR 24 for a short period of time and then enter into secondary dormancy relatively quickly.

Honiges et al. (2008) notes that there are sunflower genotypes that can be characterized as low-stimulant or germination-inhibiting towards broomrape. Wegmann (1986, 2004) and Wegmann et al. (1991) stressed the importance of phytoalexins as factors of resistance to *Orobanche*, while Sauerborn et al. (2002) did the same with benzothiadiazole (BTH).

Panchenko and Antonova (1975) concluded that the protective response of sunflower plants from different cultivars they investigated came down to the accumulation of lignin and its precompounds in injured host cells, resulting in the haustoria losing the ability to supply themselves with water and nutrients from the host cells.

Pustovoit (1966) and Honiges et al. (2008) talk of mechanical barriers as being essential to the phenomenon of broomrape resistance. Several studies by Labrousse et al. (2000, 2002, 2004) discuss different criteria for assessing *Orobanche* resistance and the different mechanisms by which such resistance operates. The authors were able to distinguish between three types of broomrape resistance in their work: 1. resistance acting at an early stage in broomrape development (*H. debilis* ssp. *debilis*), when broomrape seedlings were present on the sunflower root, but an impassable encapsulation layer blocked the intruding parasite, which then died; 2. resistance found in the resistant line LR1, which involves two types of action: i) decreased stimulation of broomrape germination (a three-fold reduction compared to susceptible line 2603); and ii) rapid necrosis that appeared as early as stage 2 of parasite development; 3.

resistance observed at a later stage of broomrape development in the line 92B6 (necrosis developing prior to broomrape flowering).

Genetic control of broomrape resistance can also be achieved by incorporating into the cultivated sunflower genes for resistance to imidazolinone-based herbicides, which are effective in controlling this parasitic weed.

CONTROLLING BROOMRAPE BY DEVELOPING IMI-RESISTANT SUNFLOWER HYBRIDS

The rapid changes in broomrape race composition have forced sunflower breeders and geneticists to not only search for genes for resistance to the new races of *Orobanche* but to also look for alternative solutions to the problem of broomrape control. In the past 10 years, the development of sunflower hybrids resistant to the imidazolinone herbicides has made it possible to successfully control broomrape regardless of its race composition.

Wild *Helianthus annuus* L. resistant to imidazolinones (imazethapyr, pursuit) was first identified in Kansas (USA) in 1996 in a soybean field treated for seven consecutive years with a herbicide from this group (Al-Khatib et al., 1998). The use of imidazolinone resistance in sunflower breeding through the introduction of IMI-resistance genes into cultivated sunflower genotypes provides a broad spectrum of weed control (covering over 40 broadleaf species and over 20 grass weed species) and is especially effective in controlling *Orobanche* in sunflower, as discovered by Alonso et al. (1998). The USDA-ARS (NDSU) research group quickly transferred this genetic resistance into cultivated sunflowers and released the public populations IMISUN-1 and IMISUN-2. Similar programs were developed in parallel by Alonso et al. (1998) in Spain, by Malidža et al. (2000) and Jocić et al. (2001) in Serbia, and by several private companies in Argentina. Bruniard and Miller (2001) reported that IMI-resistance is controlled by two genes (semi-dominant type of gene action). Imr_1 is the gene responsible for imidazolinone resistance, while Imr_2 has the modifier effect when the major gene is present. Malidža et al. (2000) and Jocić et al. (2001) showed that resistance to imidazolinones is controlled by a single, partially dominant gene. These differences in the mode of inheritance could perhaps be attributed to the presence of mutations on several different loci in the original population of wild *Helianthus annuus* L.

Imidazolinones inhibit acetolactate synthase (ALS), also called acetohydroxyacid synthase (AHAS), which is responsible for synthesizing the amino acids valine, leucine and isoleucine. Imidazolinone-tolerant plants with altered AHAS genes and enzymes have been discovered in many species (Sala et al., 2008).

Sala et al. (2008) obtained another gene for resistance to imidazolinones through ethyl methane-sulfonate mutagenesis of seeds and selection with the imazapyr herbicide. They labeled the gene CLHA-PLUS. Based on genetic analysis (F_1, F_2 and BC_1F_1), the authors determined that the IMI-resistance gene CLHA-PLUS is controlled by a partially dominant nuclear gene. Using the SSR marker for the AHASL1 gene, they concluded that the mutation present in CLHA-PLUS is different from Imr_1, but that both these genes are allelic variants of the locus $AHASL_1$.

CONCLUSIONS AND FUTURE PROSPECTS

Sunflower breeders and geneticists have been successful in responding to the rapid changes in the race composition of broomrape (*Orobanche cumana* Wallr). They found genes for resistance to this pathogen and incorporated them into elite lines of cultivated sunflower, making it possible to develop *Orobanche*-resistant hybrids. Research so far has shown that the genes for broomrape resistance are present in some wild species of the genus *Helianthus*. For this reason, it would be desirable to set up an international project that would investigate all wild sunflower species and all populations within each species for resistance to the existing populations and races of broomrape using screening on infested plots, in greenhouses, and at the molecular level. This would produce a map of genes for broomrape resistance within the genus *Helianthus*. Another international project could be set up to establish an international collection of broomrape populations (races) that would be kept within the confines of a single institution and accessible to all users. The variability of all the populations would be studied at the molecular level and a map would be developed, making it possible to develop resistant hybrids more rapidly and with a greater rate of success. It is also very important to make an effort to establish a new set of differential lines for the new races that have appeared in Russia, Romania, Ukraine, Turkey, Bulgaria, and some other countries. It is desirable that universal protocols (methods) be established for screening for resistance to broomrape in field and greenhouse conditions and at the molecular level, so that the findings of teams from different parts of the world can be compared to each other.

In parallel with the development of IMI-resistant sunflower hybrids, it is necessary to also develop new kinds of herbicides capable of controlling broomrape in addition to other weeds.

To speed up the progress of sunflower breeding for resistance to Orobanche, there should be a greater level of collaboration between the breeders from public institutions and private companies.

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Progress in breeding sunflowers for resistance to *Sclerotinia*

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ABSTRACT

Most forms of resistance to *Sclerotinia sclerotiorum* in sunflower are partial and under quantitative genetic control. Conventional breeding programmes involve field resistance tests on segregating progenies and inbred lines and field trials on potential hybrid varieties. Progress has been gradual but continuous, at rates depending on selection pressure and effort, which depend themselves on the importance of attacks in different parts of the world. With the aim of improving the rate of gain in resistance level, several types of study are in progress. QTL for resistance have been identified on all 17 linkage groups of sunflower, but with some specificities for observations of natural field attack, and with some linkage groups having greatest importance, in particular LG10. Close markers need to be developed to maker possible marker assisted selection. It would be useful to identify genes under these QTL and the first step is identification of genes involved in *Sclerotinia* resistance in *Arabidopsis* but which could also be important in sunflower. Both phenotypic and genotypic research for resistance factors in wild *Helianthus* species are required to determine whether introgressions could provide improved levels of resistance in cultivated sunflower. Many introgressions developed without selection for *Sclerotinia* resistance are highly susceptible and even the best introgressions available at present do not show levels of resistance better than the best cultivated lines. However, results suggest that some *Helianthus* species may have factors which, introduced into the best cultivated lines, could raise the level of resistance. It will be necessary to combine knowledge on QTL inheritance, resistance gene pathways and techniques for introgression of specific genes from wild species to make progress in breeding.

Key words: ascospores – capitulum – defence pathway – introgression – QTL – recurrent selection

INTRODUCTION

Sclerotinia sclerotiorum remains one of the major diseases of sunflower, with very variable attack levels, according to rainfall at particular periods of crop growth and the presence of other susceptible crops in the rotation or in neighbouring fields. Research on breeding and genetics of *Sclerotinia* resistance started about 40 years ago but although there has been considerable progress in most objectives, none can be considered as complete.

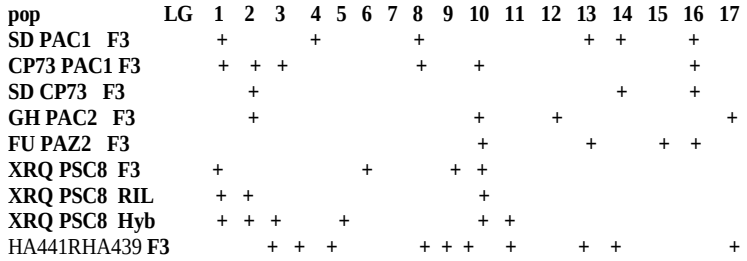
The reaction of sunflower genotypes, whether inbred lines or hybrids, can be observed satisfactorily under re-enforced natural attack or by infection with ascospore suspensions or mycelium explants. However, in all cases, plants must be grown in the field, at least until flowering, and generally almost until maturity, which is expensive and, if large scale tests are necessary, requires a large area where almost no yield will be produced. Glasshouse or growth chamber tests on seedlings, young plants or excised plant parts have not so far been used on a large scale. Research continues to optimise infection techniques, for example Gulya et al. (2008) used infected cereal grains sown with a seed drill and so possible on a very large scale.

From the first field observations in yield trials, with mean levels of attack of 20 to 60%, cultivated sunflowers showed polymorphism for reaction to *Sclerotinia* attacks on roots and stem bases (stalk rot), and capitula (head rot). However, under very severe attack, all genotypes appeared susceptible, there was no complete resistance. Terminal bud attack was rather different, since most sunflower genotypes appeared to be completely resistant, and susceptible types rather uncommon, although apparently showing favourable characteristics for yield, meaning that they could be selected in conditions without *Sclerotinia* attack. Research in the 1980s and 1990s, using infections by ascospores (natural or suspensions), mycelium explants or sclerotia, showed that resistance was partial and quantitative and mostly additive (Vear & Tourvieille, 1988). Some interactions between parental lines appeared in hybrids, but for single season results, these may have been partly due to environmental effects. These effects have made it possible to improve hybrid resistance significantly. Vear et al (2003) reported a 60% reduction in attack on varieties widely grown in France in 2000 compared with varieties that had been

developed before 1975. At Clermont-Ferrand, we carried out recurrent selection for capitulum resistance over 16 generations, with mycelial and ascospore infections and halved natural attack on hybrids and doubled the period delay before symptom appearance of inbred lines (Vear et al 2007).

The following step, to improve the efficiency of breeding programmes, and start to obtain some idea of the number of genes involved and their possible genetic linkage with other characters, was QTL analysis. Our group started this in the 1990s, soon finding a strong QTL linked to a Protein Kinase gene, apparently specific to an INRA inbred line PAC1. It explained 50% of variation in a cross with a very susceptible line and 15% in a cross with a different highly resistant line, and could have been a good candidate for control of resistance (Gentzbittel et al, 1998). However, this QTL has not been found in other populations, even when the resistance source came from recurrent selection of a population for which PAC1 was one of the constituents and when the parents showed polymorphism for the PK gene. Why this should be is not clear. Further analyses were made at INRA (Mestries et al, 1998, Bert et al, 2003, 2004, Vear et al, 2008), USDA (Yue et al, 2008) and Hohenheim University (Micic et al 2005) and also in Argentina. From the INRA and USDA work on capitulum resistance, with seven apparently different resistance sources, QTL have been identified on all seventeen linkage groups (Figure 1), the most frequent being that (or those) linked to the recessive branching gene *b1* on LG10. Two QTL, on LG 5 and 11 appear specific to hybrids observed under re-enforced natural attack. Very frequently, these QTL do not appear very significant, with lod of from 2 to 4 and, especially for those calculated for two or more years of results, each explained less than 20% of phenotypic variability. This may be due to environmental effects or to that fact that many small QTL play roles in determining resistance levels.

Fig 1. QTL for resistance to *Sclerotinia* infection of sunflower capitula



To obtain better precision, it would be necessary to observe very large progenies of RIL, in several environments or years, which would be very expensive. Thus, at present, there appear to be two methods to improve efficiency of *Sclerotinia* resistance breeding programmes. One is to search for "stronger" or at least "additional" resistance genes and the other is to obtain improved understanding of the genes already available and the processes they control to determine reaction of sunflower plants to *Sclerotinia*. With both of these objectives in mind, in 2008 and 2009 we subjected a collection of 165 cultivated sunflower lines and 103 introgressions to ascopore infection on capitula, with the aim of comparing resistance levels of many different origins to prepare association studies with genes which could be involved in resistance and have been identified elsewhere.

MATERIALS AND METHODS

Sunflower lines : A core collection of 97 cultivated sunflower lines (Coque et al, 2008), 59 INRA or public lines known for their reaction to *Sclerotinia* head rot and 103 introgression lines developed by H.Serieys, INRA, Montpellier. Resistant and susceptible controls were INRA lines SD and GU.

Two replications of 25 plants were infected in 2008 and 2009 at the beginning of flowering. .Measurements of resistance are expressed as:

1. Percentage attack (%): number of plants showing symptoms/ number of plants infected.
2. Latency Index (Ilat): duration between infection and symptom appearance / duration for mean of resistant and susceptible controls infected on the same day.
3. Resistance Index (RI): latency index*100 + (100-% attack)

RESULTS AND DISCUSSION

Cultivated sunflower lines: The graph in Figure 2 presents overall results. The resistant control, SD had a resistanxce index of 128, the susceptible, Guan RI of 72. For cultivated sunflower the mean RI of

lines not bred for resistance was 84.3, the best being restorer lines 83HR4 (148) and RHA801 (162) and maintainer line FN (158). The mean RI of lines bred for resistance was 128.3. This collection confirmed the results of Vear et al (2008), that branched lines generally show better resistance than unbranched ones, but there were a few highly susceptible branched lines, for example RIL182 (66), OQP2 (73), RHA428 (73) and SURES-2(78). RHA274 had an RI of 94 whereas the best branched lines were PSX9 (239), PSD2 (238, PSC8 (212) PST5 (206). As in 2008, we concluded that there is a genetic linkage between a QTL for resistance and the branching gene rather than a direct effect of the branching phenotype.

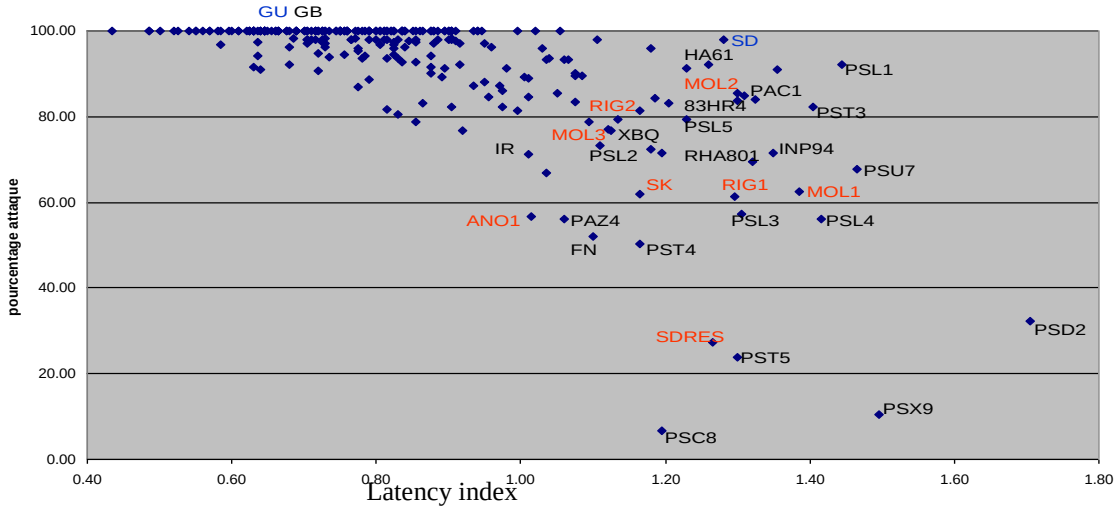


Fig. 2. Mean percentage attack and latency index for ascospore tests in 2008 and 2009 on 261 sunflower and introgression lines.

This collection also allowed us to study a factor which has been suggested to be important in *Sclerotinia* infection through florets: pollen, a possible nutrient source for *Sclerotinia*. We had one genic male sterile line, in which half the plants produced pollen and half were male sterile and 32 introgressions which segregated for restoration, such that some plants were male sterile. Overall, the latency index of the male sterile plants was slightly higher than that for male fertile plants:

2008 ms : 0.75 mf : 0.73
2009 ms : 0.92 mf : 0.87

However, when individual families are studied, there was variation in both directions and only 3 of 33 entries showed a significantly longer latency index for male sterile plants. As for branching, this variable effect may be due to the existence of QTL linked to different restorer genes. It would appear that the presence of pollen is not essential for *Sclerotinia* infection.

Wild *Helianthus* and Introgression lines: As for many diseases, wild *Helianthus* species can be expected to provide sources of resistance. Studies started at least in the 1970 and 1980, for head rot, notably by Serieys (1987), using mycelium on capitula. He found considerable differences, but with the problem that lack of fungal growth on the very small capitula that dry very quickly may not be transferable to the large heads of cultivated sunflower. In France, these tests were followed by studies of reaction to ascospores by D.Tourvieille and B.Grezes-Besset. Again, there were large differences and, in this case, some species with small capitula (*H.debilis*) appeared particularly susceptible, so the best species (*H.rigidus, H.resinosus*…) did appear to have some real resistance, although always partial. As for cultivated sunflower, tests on leaves did not give the same order as those on capitula: some cultivated Jerusalem artichoke (*H.tuberosus*) clones were the best (Tourvieille et al, 1997). For mid-stem attack, Cerboncini et al (2002) found *H.maximiliani* to be the most resistant. These difference in control has been confirmed by recent work at USDA, Lui et al (2010) reporting that factors for resistance to stalk rot may be much more widespread in wild *Helianthus* than factors for capitulum resistance.

In the recent programme, we tested 103 introgression lines developed by H.Serieys without selection for *Sclerotinia* resistance, in comparison with 2 introgression lines that had been bred for this character. Table 1 shows resistance indices for the introgression lines grouped by species. Many of the introgressions were highly susceptible, more so than the cultivated susceptible controls and none had the

resistance level of the best cultivated lines. This could be due to susceptibility of the cultivated lines used to make the introgressions (for example HA89), but also to susceptibility of the wild species itself (and there is a certain relation with results on the wild species themselves) or to loss of resistance factors during the introgression process.

Table 1. Mean Resistance Indices (RI) for introgression lines developed from several *Helianthus* species

Helianthus species	RI max	RI min	mean RI	Nb lines studied
H.mollis	175.9	54.0	94.0	12
H.rigidus	168.1	48.5	80.8	13
H.anomalus	144.9	58.0	97.8	3
H.resinosus	124.0	55.0	81.5	15
H.petiolaris	115.2	62.5	83.4	7
H.strumosus	106.7	76.0	88.8	7
H .tuberosus	102.5	60.0	80.5	11
H.exilis	97.5	64.0	79.3	4
H.debilis	90.7	63.0	73.2	10
H.bolanderi	89.4	48.5	72.7	4
H.neglectus	79.6	72.5		2
H.occidentalis			82.5	1
H.decapetalus			82.2	1
H.deserticolar			78.5	1
H.niveus		66.5	1	
H.argophyllus		66.0	1	

However, there was wide diversity between introgressions from some species and it is notable that some introgressions from *H.mollis, H.rigidus, H.resinosus* and perhaps *H.anomalus* can be classed as having good resistance whereas others are highly susceptible. These are the species which can be suggested as having the most promise for improving *Sclerotinia* resistance levels. We did not test any introgressions from *H.maximiliani*, so cannot make any comparison with the work of Rönicke et al (2004). Feng et al (2009) and Lui et al (2010) also reported interesting progenies from *H.maximiliani* and *H.nutalli.*

In comparison with the "*Sclerotinia*-blind" introgressions from H.Serieys, we studied 2 pairs lines, a susceptible cultivated lines, PW3, and its more downy mildew resistant form PW3RM and a resistant cultivated line SD, compared with lines selected for resistance after pollination with a sunflower x H.resinosus cross : RESPW3 and SDRES. The RESPW3 showed improvement mainly in latency index (91 compared with 83 for PW3 and 75 for PW3RM). In contrast, SDRES had a very similar latency index to SD (127 and 128 respectively) but a much lower percentage attack (27% compared with 98%). SD is our resistant control and SDRES was the best unbranched maintainer line in the collection studied.

From these results, it may be suggested that introgressions bred for *Sclerotinia* resistance must be considered in terms of additional, "supplementary" genes compared with those in cultivated sunflower. SD was already a good line for latency index, so interesting genes were those that reduced % infection. The *Helianthus* species with the highest level of resistance may not necessarily be those that which will give the best improvement of cultivated sunflower. It will be particularly important to be able to follow specifically the introduction of interesting genes. Rönicke et al (2004) reported following fragments of *H.maximiliani* genome during an introgression programme by AFLP, but without knowing whether interesting genes from the wild species were being retained. Liu et al (2010) used in situ hybridization (GISH) to follow chromosomes or segments of chromosomes from wild species, but it is still not possible to know whether the required genes are retained.

Identification of genes involved in Sclerotinia resistance: From these results, it is clear that, both to improve introgression from wild *Helianthus* and to obtain close markers for QTL, knowledge of the genes involved must be improved

For sunflower, soybean, lettuce, tomato and oilseed rape, an oxalate oxidase (OXO) gene has been introduced from barley, wheat or another fungal species by genetic engineering. It gave reductions of symptoms in every crop and in particular sunflower (Hu et al. 2003). However, since GMO sunflowers are not, or very little developed, it would be interesting to know whether variation for this gene or its regulation occurs in sunflower or its wild relatives. It is not clear whether this OXO gene is the reason for

no *Sclerotinia* attack on cereals. It would be useful to have information on whether there are definite pathways which make attack impossible in *Poaceae.*

The wide host range of *Sclerotinia* has the disadvantage that small differences between sunflower genotypes do not stop the pathogen, but it also means that genes known in one species may also operate in another. In particular, work in progress on reaction of *Arabidopsis* mutants could give some ideas of the genes and enzyme pathways involved. Guo and Stotz, (2007) reported that jasmonic and absissic acid pathways are involved in defence reactions. Perchpied and Balagué et al (2010) developed tests to determine the reaction of this model species to *Sclerotinia*. Because it is highly susceptible, they used an oxalate-deficient pathogen strain to make possible differentiation between genotypes carrying mutations for known genes. They confirmed that inducible defences mediated by jasmonic acid, absissic acid and ethylene pathways are important and showed that that NO and ROS are essential signals. Reaction to *Sclerotinia* appears independent of salicylic acid, logically since this pathway often appears involved in reactions to obligate parasites. The last authors also showed that the gene sequences involved are conserved in rapeseed, another crucifer. It now remains to be tested whether these genes are conserved in sunflower, whether they show polymorphism between resistant and susceptible lines and if they map in QTL regions.

Other differences in metabolism have been reported between sunflowers with different levels of resistance to *Sclerotinia* when they are infected with the pathogen. Peluffo et al (2009) investigated the primary carbon metabolism directly in florets infected with *Sclerotinia* ascospores and found that, on infection, there were greater changes in the susceptible genotype HA89 than in the more resistant RHA801. They suggested that different specific regulatory genes were involved. Giacomelle et al (2010) reported differences in WRKY proteins after mycelium infection of HA89 and HA853 leaves and suggested that these proteins could be markers of partial resistance.

CONCLUSIONS

With the variability of intensity of attack by *Sclerotinia* according to weather conditions, it is difficult to forecast how important stalk and head rots will be in the future. Climate change may reduce rainfall in periods in which sunflowers are in flower at present, but yield will be lost also. There are already studies to determine if sunflowers can be sown earlier in spring, or even as an autumn crop, and this would again increase the likelihood of *Sclerotinia* attack. Generally, conditions good for *Sclerotinia* are also good for yield. Thus, the slow but continued progress in resistance in sunflowers is likely to continue to be of use for the crop in many areas of the world. It will be necessary to combine knowledge on QTL inheritance, resistance gene pathways and techniques for introgression of specific genes from wild species to make progress in breeding.

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Utilization of wild *Helianthus* species in breeding for disease resistance

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ABSTRACT

There are 51 species of wild *Helianthus*, 14 annual and 37 perennial. The genus *Helianthus*, besides constituting the basic genetic stock from which cultivated sunflower originated, continues to contribute characteristics for cultivated sunflower improvement. In a recent survey of 13 major crops for introduced genes from wild ancestors, sunflower ranks fifth with seven traits. It is estimated that wild species contribute 270 to 385 million USD per year to just the USA's sunflower industry. Much of this value is derived from resistance genes for several major diseases including rust, downy mildew, Verticillium wilt, powdery mildew, Phomopsis stem canker, Sclerotinia wilt, charcoal rot, Phoma black stem, and the parasitic weed broomrape. The frequency of downy mildew resistance genes in the wild annual species is high with dominant genes controlling single specific races being the most common, while multiple race resistance to all known races has been identified in only two populations of *H. argophyllus*. The frequency of rust resistance genes is also high in wild annual species with many populations containing rust-resistant plants, but immunity or total susceptibility is rarely found. *Helianthus tuberosus* has been a source of disease resistance genes for over 50 years. It has been particularly useful for stem-infecting diseases such as Sclerotinia stalk rot, Phomopsis stem canker, Phoma black stem, and charcoal rot. The perennial species also have provided resistance genes for broomrape, where most of the perennial species have been reported to have immunity to the parasite. Significant progress has been made in collecting and preserving the wild *Helianthus* species germplasm, but only a small portion of the available genetic diversity has been exploited for sunflower improvement. This germplasm will be important in the future as new races of diseases evolve and new diseases appear.

Key Words: broomrape – disease resistance – downy mildew – genetic resources – *Helianthus* – rust

INTRODUCTION

Crop wild relatives, which include the progenitors of crops, as well as other species more or less closely related to them, have been undeniably beneficial to modern agriculture, providing plant breeders with a broad pool of potentially useful genes (Holden et al. 1993; Hajjar and Hodgkin 2007). Wild relatives of crop plants typically are genetically much more diverse than related cultivated lineages. Genetic diversity contributes to long-term survival of species by allowing them to adapt quickly to changes in their environment.

The genus *Helianthus*, besides constituting the basic genetic stock from which cultivated sunflower originated, continues to contribute specific characteristics for cultivated sunflower improvement. Genetic diversity is critical to successful crop breeding programs, but to date its exploitation has been rather limited (Harlan 1976). This has been the case with cultivated sunflower where the wild species of *Helianthus* have been used to a limited extent, but have a tremendous amount of genetic diversity that could be exploited. The genetic diversity of the wild species can make a significant contribution to the global sunflower industry by providing genes for resistance (tolerance) to pests and environmental stresses, allowing the crop to become and remain economically viable.

The wild sunflower species are adapted to a wide range of habitats and possess considerable variability for most agronomic and achene quality characters, and for their reaction to insects and disease pathogens. Hajjar and Hodgkin (2007) surveyed introduced genes in 13 crops of major importance to global food security from the mid-1980s to 2005 and reported that sunflower had seven contributed traits, the fifth highest of the crops surveyed. The estimated economic contribution of the wild species to the cultivated sunflower in the USA is $384 million per year (Prescott-Allen and Prescott-Allen 1986). Another estimate is $269.5 million per year (Phillips and Meilleur 1998). The greatest value was attributed to the genes for resistance to pathogens. Wild *Helianthus* species have been a reliable source of genes for resistance to economically important pathogens. Much of the value is derived from disease resistance genes for rust, downy mildew, Verticillium wilt, Alternaria leaf spot, powdery mildew, Phomopsis stem canker, Sclerotinia wilt/rot, and the parasitic weed broomrape. When screening wild

sunflower species as potential sources of genes, it should be realized that plants within a single population of a species may exhibit different levels of resistance to a given pest due to segregation, since the native populations are open-pollinated and segregating for many traits. It is also important to consider more than one population of a species when characterizing resistance genes from a single species. There is a continued need to collect, maintain, evaluate and enhance wild *Helianthus* germplasm for future improvement of cultivated sunflower.

DISCUSSION

Diseases limit production in a majority of sunflower-producing countries. Sunflower is a host to a wide array of diseases that can cause serious economic damage in terms of yield and quality, with the fungal diseases the most numerous and economically serious. In the USA, the major diseases of concern are downy mildew, rust, Sclerotinia head and stalk rot, and Phoma black stem. Verticillium wilt, Phomopsis stem canker, Alternaria leaf spot, Septoria leaf spot, charcoal stem rot, and Rhizopus head rot occur to a lesser degree. In Europe and adjacent Mediterranean countries, downy mildew, Sclerotinia head rot, Phomopsis, Botrytis gray rot, and charcoal rot are considered the most important diseases. Some diseases are important in only a few countries, such as Verticillium wilt in Argentina and white rust (*Albugo*) in South Africa. Wild sunflower species have been a valuable source of resistance genes for many of the common pathogens of cultivated sunflower. The relative severity of individual diseases varies widely, depending on climate and host cultivars. Breeding for resistance often is the most effective means of control. Sources of resistance or improved levels of tolerance for most diseases are available among the cultivated sunflower and the wild species of *Helianthus*.

Downy Mildew

Downy mildew, caused by *Plasmopara halstedii* (Farl.) Berl. and de Toni, occurs in countries where sunflower is grown, with the exception of Australia. Among the methods of control, host-plant resistance using race-specific genes designated *Pl*, of which 18 have been described, is the most effective (Gulya 2007). The constant evolution of new physiological races, due to pathogenic variability and selection pressure resulting from the use of resistant hybrids and seed treatment fungicides, continuously challenges breeders to identify and introduce new resistance genes or gene clusters. Wild sunflower species have been a plentiful source of genes for downy mildew resistance.

Downy mildew can be controlled by single, race-specific major dominant genes. Multi-race resistant germplasm and single-race resistant germplasms from wild sunflower species have been developed (Miller and Gulya 1988; Tan et al. 1992; Jan et al. 2004b). Wild *H. annuus*, *H. petiolaris*, *H. tuberosus*, and *H. praecox* ssp. *runyonii* are sources of dominant genes for single race resistance, while *H. argophyllus* is the only known source of dominant genes for all current races of the fungus (Vrânceanu and Stoenescu 1970; Zimmer and Kinman 1972; Miller and Gulya 1988, 1991; Pustovoit and Krokhin 1977; Seiler 1991c; Tan et al. 1992; Miller et al. 2002; Dussle et al. 2004; Jan et al. 2004b; Hulke et al. 2010).

Complete resistance to downy mildew has been reported in annual species *H. annuus*, *H. argophyllus*, *H. debilis*, and *H. petiolaris*, and perennial *H. decapetalus*, *H. divaricatus*, *H. eggertii*, *H. giganteus*, *H.* x *laetiflorus*, *H. mollis*, *H. nuttallii*, *H. scaberrimus*, *H. pauciflorus*, *H. salicifolius*, and *H. tuberosus* (Christov 1996b). Diploid perennial species *H. divaricatus*, *H. giganteus*, *H. glaucophyllus*, *H. grosseserratus*, *H. mollis*, *H. nuttallii*, and *H. smithii* and their interspecific hybrids were resistant to downy mildew (Nikolova et al. 1998). Interspecific hybrids based on *H. eggertii* and *H. smithii* were resistant to downy mildew in Bulgaria (Christov et al. 1998). Among annual species screened at the Veidelevka Institute of Sunflower, Veidelevka, Russia, high resistance to downy mildew was observed in populations of *H. argophyllus*, *H. niveus*, *H. neglectus*, *H. debilis*, and *H. petiolaris* in field screening (Tikhomirov and Chiryaev 2005).

Helianthus argophyllus-derived germplasm ARG-1575-2 carries the Pl_{arg} locus conferring resistance to all known races of downy mildew. Since Pl_{arg} was mapped to a linkage group different from all other *Pl* genes previously mapped using SSRs, it can be concluded that Pl_{arg} provides a new unique source of resistance to downy mildew (Dussle et al. 2004; Wieckhorst et al. 2008). Plants resistant to downy mildew were found in populations of wild *H. annuus* and *H. argophyllus* with the percentage of resistant plants in *H. annuus* accessions ranging from 9 to 100%, and 50 to 58% in *H. argophyllus* (Terzić et al. 2007).

Christov (2008) reported that 850 interspecific hybrid combinations based on nine annual species and 27 perennial species were resistant to downy mildew in a field screening in Bulgaria. This indicated that

the resistance genes for downy mildew are widespread within the genus *Helianthus*. Resistance to downy mildew was reported by Nikolova et al. (2004) in different progenies of interspecific hybrids with *H. pumilus*. Other reports of wild species as potential sources of resistance to one or more races of downy mildew include annual species *H. paradoxus*, *H. deserticola*, and *H. praecox* ssp. *hirtus*, and perennials *H. grosseserratus*, *H. maximiliani*, *H. nuttallii*, and *H. pauciflorus* (Fick et al. 1974; Thompson et al. 1978; Seiler 1991a, b). Two cultivars based on wild *H. annuus* and *H. tuberosus*, 'Progress' and 'Novinka', have been developed from the "group immunity" cultivars developed by Pustovoit et al. (1976). Complete resistance to downy mildew was reported in interspecific hybrids with *H. salicifolius* by Encheva et al. (2006b). Christov et al. (1996b) indicated that wild *H. petiolaris* had resistance genes to downy mildew, while Tarpomanova et al. (2009) indicated that interspecific hybrids based on *H. bolanderi* had resistance to races 330 and 700 of downy mildew. The multitude of genes from the wild species for downy mildew resistance is supported by the number of germplasm releases that incorporate protection against ever-evolving pathotypes of downy mildew that infect cultivated sunflower.

Sclerotinia

Sunflower stalk and head rot (white mold) incited by *Sclerotinia sclerotiorum* (Lib.) de Bary is considered the most devastating disease of sunflower in many parts of the world. Sclerotinia wilt causes the greatest losses to sunflower on a global basis. This is in part due to the wide host range of *Sclerotinia sclerotiorum*, a facultative parasite that attacks 360 species of plants. Cultivated sunflower is highly susceptible to this pathogen whose attack is manifested in several forms, including destruction of the root, stem, head, and seeds. Since no cultural practices or effective fungicides are available to control the disease, efforts are being made to develop resistant or tolerant hybrids. It appears that Sclerotinia resistance is complex, involving many genes, each with small effects. This requires different breeding strategies than those used for simply inherited resistance controlled by single or a few genes.

Sclerotinia stalk and head rot can reduce seed yields more than 50 percent, causing serious economic losses. Cultivated sunflower generally lacks resistance to Sclerotinia, although some differences in susceptibility exist. However, the over 51 species of *Helianthus*, consisting of diploids, tetraploids, and hexaploids, is a diverse pool of potential sources of Sclerotinia resistance. Evaluation of wild germplasm indicated that several wild perennial species possess high levels of resistance to Sclerotinia head rot and stalk rot.

Sclerotinia head rot tolerance was observed in perennials *H. resinosus*, *H. tuberosus*, *H. decapetalus*, *H. grosseserratus*, *H. nuttallii*, and *H. pauciflorus* (Pustovoit and Gubin 1974; Mondolot-Cosson and Andary 1994; Ronicke et al. 2004). Certain wild species of sunflower or their interspecific progenies crossed with *H. annuus* have high levels of resistance to *S. sclerotiorum* (Christov et al. 1996b; Cerboncini et al. 2002, 2005; Rashid and Seiler 2004, 2006). Cáceres et al. (2006) reported that some populations of *H. petiolaris* naturalized in Argentina had smaller lesions on leaves caused by *S. sclerotiorum* than others, but that stem lesions were not significantly different. Interspecific crosses with *H. argophyllus* were reported to have high tolerance to Sclerotinia head rot (Christov et al. 2004). Among the perennial species, resistance to Sclerotinia was observed in populations of *H. tuberosus*, *H. divaricatus*, *H. hirsutus*, *H. maximiliani*, *H. mollis*, *H. nuttallii*, *H. occidentalis*, and *H. rigidus* (=*pauciflorus*) grown under natural infection conditions (Tikhomirov and Chiryaev 2005). Block et al. (2009) reported that in greenhouse screening, *H. argophyllus* (PI 650078) had 94% of the plants resistant to Sclerotinia stalk rot.

Among field-screened annual species at the Veidelevka Institute of Sunflower, Veidelevka, Russia, high resistance to Sclerotinia was observed in populations of *H. argophyllus*, *H. niveus*, *H. neglectus*, *H. debilis*, and *H. petiolaris* (Tikhomirov and Chiryaev 2005). Hahn (2002) reported that interspecific lines based on *H. argophyllus* and *H. praecox* ssp. *runyonii* were the most head rot resistant.

Christov (1996b) reported that higher ploidy perennial species (hexaploid and tetraploid species) exhibited greater susceptibility than the diploids, with *H. glaucophyllus*, *H. divaricatus*, *H. salicifolius*, and *H. mollis* having the highest frequency of healthy plants. Tolerance to Sclerotinia was observed in the perennials *H. eggertii*, *H. pauciflorus*, and *H. smithii*, and annuals *H. annuus*, *H. argophyllus*, *H. petiolaris*, and *H. praecox* (Christov 2008). Interspecific hybrids with perennial *H. maximiliani* exhibited higher levels of stalk rot resistance than head rot-resistant inbred lines (Cerboncini et al. 2002; Ronicke et al. 2004). Rashid and Seiler (2004, 2006) identified potential sources of Sclerotinia head and stem rot resistance in populations of perennial *H. maximiliani* and *H. nuttallii* ssp. *rydbergii* from Canada. Škorić and Rajčan (1992) reported that *H. maximiliani* possessed a high degree of resistance to Sclerotinia. Perennial *H. resinosus* has been identified as a source for resistance to Sclerotinia head rot by Mondolot-

Casson and Andary (1994). Block et al. (2009) reported that populations of *H. resinosus*, PI 650079 and PI 650082, had 100% resistant plants in greenhouse screening.

Some progress has been made in increasing the resistance to Sclerotinia stalk rot in cultivated sunflower. Sclerotinia stalk rot tolerance was observed in annual *H. praecox*, and perennials *H. pauciflorus*, *H. giganteus*, *H. maximiliani*, *H. resinosus*, and *H. tuberosus* (Škorić 1987). Kohler and Friedt (1999) indicated that progenies of interspecific crosses with *H. mollis* and *H. tuberosus* had increased levels of tolerance to white mold stalk infection. Stalk rot resistance was reported by Micic et al. (2004) in an interspecific cross with *H. tuberosus* they used to determine QTLs for resistance. The original interspecific cross was described by Degener et al. (1999a). Lines derived from *H. argophyllus* and *H. tuberosus* had the lowest Sclerotinia stem lesions in a study by Degener et al. (1999b). Interspecific hybrids based on *H. nuttallii*, *H. giganteus*, and *H. maximiliani* were reported to show resistance against stem infection by Henn et al. (1997). Miller and Gulya (1999) developed four maintainer and four restorer oilseed lines with improved tolerance to Sclerotinia stalk rot. Inbred line HA 410 released by Miller and Gulya (1999) derived from a wild perennial hexaploid, *H. pauciflorus* (=*rigidus*), had moderate tolerance to stalk rot. Sclerotinia root rot tolerance was observed in perennials *H. mollis*, *H. nuttallii*, *H. resinosus*, and *H. tuberosus* (Škorić 1987).

A program focusing on the transfer of Sclerotinia stalk rot resistance from wild *Helianthus* species of different ploidy levels (2x, 4x, 6x) into adapted sunflower germplasm via interspecific hybridization was initiated by the Sunflower Research Unit in Fargo in 2002 (Jan and Seiler 2008). Hexaploid perennial *H. californicus*, which had been identified as highly resistant to Sclerotinia stalk rot, was crossed with the moderately tolerant line HA 410 (Miller and Gulya 1999) followed by continuously backcrossing with HA 410 until BC_4F_1, and the chromosome number of the BC progeny was reduced to 2n=34 (Feng et al. 2006, 2007a).

Interspecific amphiploids derived from *H. divaricatus*, *H. grosseserratus*, *H. maximiliani*, *H. nuttallii*, and *H. strumosus* were identified that segregated for high levels of resistance to Sclerotinia stalk rot, helping to expand the diversity of resistance genes. These amphiploids were crossed with HA 410 and further backcrossed twice to transfer stalk rot resistance (Jan et al. 2006; Feng et al. 2007b, 2008, 2009).

Transfer of Sclerotinia stalk rot resistance from three diploid perennial species, *H. maximiliani*, *H. giganteus*, and *H. grosseserratus*, produced only 155 BC_1F_1seeds from 64,618 florets pollinated with HA 410. This result was consistent with the conclusion that diploid perennial species could be crossed with cultivated sunflower, but the frequency of successful crosses was low (Atlagić et al. 1995). The extremely low backcross seed set of the F_1 plants is the most limiting factor for transferring genes from diploid perennials. However, since the F_1 plants are generally perennial, sufficient BC_1F_1 seeds can be obtained by repeated pollination (Jan and Seiler 2008).

Sclerotinia sclerotiorum generates substantial quantities of oxalic acid, which has been identified as one of the key components in the infection process. One strategy for resistance is to produce transgenic plants that degrade the free oxalic acid. A wheat (*Triticum aestivum* L.) oxalate oxidase gene (*OXO*) has been transferred into sunflower via transformation (Scelonge et al. 2000). A transgenic sunflower line, *H. annuus* cv. SMF3, constitutively expressed the wheat *OXO* gene (Hu et al. 2003) and exhibited enhanced resistance against the oxalic acid-generating Sclerotinia fungus. The line used in the transformation event, SMF3, was originally released by Rogers et al. (1984) as a sunflower moth-tolerant germplasm based on an interspecific hybrid using *H. petiolaris*. Use of a transgenic trait for Sclerotinia control in sunflower awaits further testing and commercialization.

In conclusion, potential interspecific pre-breeding Sclerotinia resistance lines from diploid, tetraploid, and hexaploid germplasm have been produced. Evaluation of these pre-breeding lines for their reaction to Sclerotinia stalk and head rot will verify the effectiveness of the different approaches for the selection of QTLs. The effectiveness of using each of the different approaches will also be verified by tracking of the wild species' specific molecular markers in progeny plants when they first reach the 2n = 34 stage and are ready for seed increase for field evaluation. The ultimate goal is to release germplasms with improved resistance to Sclerotinia stalk and head rot as soon as possible.

The Sclerotinia disease complex appears to be very complicated. The prospect of finding a single dominant gene for resistance does not look promising, but progress is being made in the development of germplasm with increased tolerance to Sclerotinia head and stalk rot. Currently there are no commercial hybrids which possess a satisfactory level of resistance to Sclerotinia rot.

Rust

Sunflower rust, a foliar disease caused by *Puccinia helianthus* Schwein. occurs in almost all sunflower growing regions. Wild *Helianthus* species have been an important source of rust resistance genes for

cultivated sunflower for several years. Resistance genes R_1 and R_2, which have been widely used in sunflower breeding programs, originated from outcrosses with wild sunflower in Texas and are believed to be the first host-plant resistance genes used in cultivated sunflower production (Putt and Sackston 1957, 1963). Hennessy and Sackston (1972) concluded that most species of wild sunflower in Texas were heterogeneous for rust resistance. An extensive survey of over 200 populations of seven species including two annual species, *H. annuus* and *H. petiolaris*, and five perennial species, *H. maximiliani*, *H. nuttallii*, *H. grosseserratus*, *H. pauciflorus* (*rigidus*), and *H. tuberosus* from the north central USA was undertaken by Zimmer and Rehder (1976). Plants free of rust were observed in 190 of 200 populations of wild annual and perennial *Helianthus* species. They observed a considerable variability in rust resistance, with widespread resistance in wild annual populations from Nebraska and Kansas. Quresh et al. (1993) and Quresh and Jan (1993) observed that resistance to rust races 1, 2, 3, and 4 in 78 populations of *H. annuus*, *H. argophyllus*, and *H. petiolaris* was 25, 28, 15 and 26% of all plants for the four races, respectively. Only 10% of the plants were resistant to all four races of rust. McCarter (1993) observed varying levels of resistance to rust in populations of *H. tuberosus*. Immunity to rust has been reported in lines derived from *H. tuberosus* (Pogorietsky and Geshle 1976). Resistance to the prevailing North American races of rust has been identified in three wild annual species, *H. annuus*, *H. petiolaris*, and *H. argophyllus* (Jan et al. 2004a).

It appears that most wild populations contain some rust-resistant plants, but complete resistance or total susceptibility of populations is rare. Since *P. helianthi* races are host specific, susceptible wild *Helianthus* species provide selective hosts in which virulent races of rust develop. Because rust races are continually evolving, it is necessary to have new genetic sources of resistance available. The rust pathogen can be effectively controlled in sunflower for long periods of time through the use of specific race genes found in the wild species. Genes for rust resistance are frequent in the wild progenitors of the cultivated sunflower (Quresh et al. 1993). In most cases rust resistance appears to be conditioned by a single dominant gene.

Phomopsis

Phomopsis brown stem canker, caused by *Diaporthe helianthi*/*Phomopsis helianthi* Munt.-Cvet. et al., was first discovered in sunflower in Yugoslavia in 1980 and is now recognized as a serious problem in much of Europe (Mihaljcevic et al. 1982; Acimovic 1984; Škorić 1985). It has subsequently become a serious threat in North and South America (Virányi 2008). Soon after its appearance, it became the most limiting factor for sunflower production in many parts of Europe, including the former Yugoslavia, Romania, Hungary, and France. Cuk (1982) reported that wild annual *H. debilis* and perennial *H. pauciflorus* are potential sources of resistance to *P. helianthi*. Kurnik and Walcz (1985) reported resistance to Phomopsis in annual *H. argophyllus*, tolerance in two other wild species, and susceptibility in local populations of perennial *H. tuberosus*. Škorić (1985) also reported tolerance in four inbred lines: two based on perennial *H. tuberosus*, and one each based on annual *H. annuus* and *H. argophyllus*. Dozet (1990) observed a high degree of resistance in two populations of *H. tuberosus*. Phomopsis brown stem canker resistance has been found in perennials *H. maximiliani*, *H. pauciflorus*, *H. hirsutus*, *H. resinosus*, *H. mollis*, and *H. tuberosus* (Škorić 1985; Dozet 1990). Cultivated hybrids developed from *H. tuberosus* and *H. argophyllus* have high field-tolerance to Phomopsis brown stem canker (Škorić 1985). Škorić (1985) hypothesized that the resistance was controlled by two or more complementary genes, and that resistance was associated with the "stay green" stem character, and with resistance to charcoal rot, Phoma black stem, and drought. Nikolova et al. (2004) reported resistance to stem canker in progenies of interspecific hybrids with perennial *H. pumilus*. Resistance to Phomopsis was reported in interspecific hybrids derived from *H. argophyllus*, *H. deserticola*, *H. tuberosus*, and *H.* x *laetiflorus* (Degener et al. 1999b).

Among annual species, high resistance to Phomopsis brown stem canker was discovered in populations of *H. argophyllus*, *H. niveus*, *H. neglectus*, *H. debilis*, and *H. petiolaris* in field screening at the Veidelevka Institute of Sunflower, Veidelevka, Russia (Tikhomirov and Chiryaev 2005). Among the perennial species, resistance to Phomopsis was observed in populations of *H. tuberosus*, *H. divaricatus*, *H. hirsutus*, *H. maximiliani*, *H. mollis*, *H. nuttallii*, *H. occidentalis*, and *H. rigidus* (= *pauciflorus*) grown under natural infection conditions (Tikhomirov and Chiryaev 2005). The perennial species *H. mollis*, *H. nuttallii*, *H. resinosus*, *H. hirsutus*, *H. divaricatus*, *H. rigidus* (= *pauciflorus*), *H. tuberosus*, *H. strumosus*, *H. decapetalus*, *H. maximiliani*, *H. smithii*, *H. doronicoides*, *H. orygalis*, and *H.* x *multiflorus* possess high levels of resistance to Phomopsis, which makes them suitable donors for resistance (Encheva et al. 2006a). Interspecific hybrids based on *H. eggertii* and *H. smithii* showed high tolerance to Phomopsis in Bulgaria (Christov et al. 1998). Cáceres et al. (2007) observed the variability in brown necrotic responses

on leaves and stems of annual *H. petiolaris* after inoculation with *Diaporthe*. Some half-sib families had significant tolerance to stem canker. Christov (2008) identified annuals *H. annuus, H. argophyllus*, and *H. debilis*, and perennials *H. pauciflorus*, *H. laevigatus*, *H. glaucophyllus*, and *H. eggertii* as potential sources of Phomopsis brown stem canker resistance, based on field screening in Bulgaria. Among the perennial wild sunflowers, diploid *H. divaricatus* was identified as a source of tolerance to Phomopsis (Korrell et al. 1996). Škorić (1987) reported that *H. salicifolius* had resistance to Phomopsis. Complete resistance to Phomopsis was reported in interspecific hybrids with *H. salicifolius* by Encheva et al. (2006b) and (Škorić (1992b).

Verticillium

Verticillium wilt, an important disease of cultivated sunflower caused by the soilborne fungus *Verticillium dahliae* Kleb., infects sunflower roots, causing wilting and leaf mottling. This disease is especially severe in Argentina. The disease can be controlled by single resistance genes; however, virulent strains of Verticillium wilt have been identified recently, prompting a search for additional sources of resistance. Resistance to *V. dahliae* is widespread in wild sunflowers. *Helianthus annuus*, *H. petiolaris*, and *H. praecox* were the major sources of genes (V-1) for Verticillium wilt resistance prior to the recent identification of a new strain. Hoes et al. (1973) tested 40 populations of wild *H. annuus* and *H. petiolaris* from six locations in Manitoba and Saskatchewan, Canada, and from 40 locations in 12 states of the USA. There seemed to be a greater frequency of resistant plants in populations from more southerly latitudes, suggesting the existence of a center for resistance to Verticillium wilt in the general area postulated to be the center of origin (southwestern USA) for wild *H. annuus*. Putt (1964) discovered a source of resistance to *V. dahliae* in line CM144, which was derived from an interspecific hybrid with wild *H. annuus.* This germplasm was used to produce released inbred lines (Fick and Zimmer 1974). Only slight disease symptoms were reported on *H. hirsutus*, *H. occidentalis*, and *H. tuberosus*, while populations of *H. resinosus* were free of the disease (Škorić 1984). Pustovoit et al. (1976) reported that all wild *Helianthus* species, except perennial *H. tomentosus* Michx., are susceptible to Verticillium wilt.

A new North American strain of *Verticillium dahliae* from North Dakota and Minnesota, USA was identified in 2004 (Gulya 2004; Radi and Gulya 2006). The new strain is able to overcome the single dominant V-1 resistance gene used in oilseed and confectionery sunflower. Further study indicated that the new biotype was also present in Manitoba, Canada. Screening cultivated germplasm has begun (Radi and Gulya 2006). It is highly likely that the wild species will be a source of resistance genes for this new strain based on past experience with the V-1 gene from wild *H. annuus*. Since there is no agronomically feasible chemical, biological, or cultural control of Verticillium on sunflower, the search for resistance to the new strain in either cultivated or wild germplasm is imperative.

Phoma

Phoma black stem, caused by *Phoma macdonaldii* Boerma, is present in all sunflower-producing regions of the world. In some regions, including the USA, the disease appears late in the season, resulting in very little economic losses. Although Phoma black stem occurs in several European countries, the disease is severest in France where basal stem lesions often result in lodging (Virányi 2008). The pathogen appears as black spots on stems and occasionally on leaves of affected plants. So far, most sunflower genotypes are susceptible to the pathogen. It has been suggested that resistance could be associated with the "stay green" stem character, and with resistance to Phomopsis stem canker. Under natural infection, wild sunflower species *H. maximiliani*, *H. argophyllus*, *H. tuberosus*, and *H. pauciflorus* possess excellent resistance to Phoma black spot (Škorić 1992a, b). Phoma black stem resistance has been reported in several perennial species, *H. decapetalus, H. eggertii*, *H. hirsutus*, *H. resinosus*, and *H. tuberosus* (Škorić 1985). Tolerance to Phoma was reported in interspecific hybrids with *H. salicifolius* by Encheva et al. (2006b). Interspecific sunflower germplasms resistant to Phoma have been developed recently using *H. eggertii*, *H. laevigatus*, *H. argophyllus*, and *H. debilis* (Christov 2008).

Under natural infection, wild *H. maximiliani*, *H. tuberosus*, *H. pauciflorus*, and *H. argophyllus* possessed excellent resistance to Phoma black stem (Škorić 1992a, b). Complete resistance to Phoma black stem was reported in annual *H. debilis* ssp. *silvestris* and perennial *H. glaucophyllus* (Christov 1996a). According to Škorić (1985, 1992a, b), resistance to Phoma black stem is positively correlated with resistance to Phomopsis and charcoal rot.

Alternaria

Alternaria leaf spot, caused by *Alternaria helianthi* (Hansf.) Tub. and Nish., causes losses in cultivated sunflower in the USA and other parts of the world. In warm climates with high rainfalls, it causes

defoliation and reduces yield significantly (Sackston 1981). All 21 annual taxa and 18 of 21 perennial species evaluated were susceptible to *A. helianthi* using applied spore suspensions, while perennial species *H. hirsutus*, *H. pauciflorus* ssp. *subrhomboideus*, and *H. tuberosus* appear to resist infection by *A. helianthi* (Morris et al. 1983). Lipps and Herr (1986) examined 13 accessions of *H. tuberosus* with significantly less Alternaria leaf spot than commercial hybrids. They concluded that *H. tuberosus* is a potential source of resistance to leaf spot. Several wild annual species, *H. praecox*, *H. debilis* ssp. *cucumerifolius*, and *H. debilis* ssp. *silvestris*, had high levels of resistance to Alternaria leaf spot and Septoria leaf spot caused by *Septoria helianthi* Ell. & Kell. in field evaluations (Block 1992). Moderate levels of resistance to leaf spot have been reported in *H. argophyllus* by Anilkumar et al. (1976).

Nine perennial *Helianthus* species, *H. maximiliani*, *H. mollis*, *H. divaricatus*, *H. simulans*, *H. occidentalis*, *H. pauciflorus*, *H. decapetalus*, *H. resinosus*, and *H. tuberosus* were highly resistant to *Alternaria* leaf spot; all annuals were susceptible (Korrell et al. 1996; Sujatha et al. 1997). Madhavi et al. (2005) found *Helianthus occidentalis* and *H. tuberosus* to be highly resistant, while *H. hirsutus* was moderately resistant. Tolerance to Alternaria leaf spot was observed in interspecific derivatives of *H. divaricatus* and cultivated sunflower (Prabakaran and Sujatha 2003). Sujatha and Prabakaran (2006) transferred Alternaria resistance from two hexaploid species, *H. resinosus* and *H. tuberosus* into diploid cultivated sunflower using anther culture. In vitro screening of parents, interspecific hybrids, and anther culture plantlets showed that 65.5% of the anther-cultured plants of interspecific hybrids from *H. tuberosus* and 24.8% of the anther-cultured plants derived from interspecific hybrids involving *H. resinosus* were resistant to *A. helianthi*. Under field conditions in India, no symptoms of Alternaria leaf spot were observed on *H. simulans* or its interspecific hybrids (Prabakaran and Sujatha 2004). Christov (2008) reported that perennial *H. decapetalus*, *H. laevigatus*, *H. glaucophyllus*, and *H. ciliaris* were potential sources of genes for Alternaria resistance based on field screening in Bulgaria. Škorić (1987) reported that *H. salicifolius* had resistance to Alternaria. Complete resistance to Alternaria leaf spot was reported in interspecific hybrids with *H. salicifolius* by Encheva et al. (2006b).

Powdery Mildew

Powdery mildew, caused by *Erysiphe cichoracearum* DC. ex Meret, is a common foliar disease on senescing leaves of cultivated sunflower in warm regions of the world (Zimmer and Hoes 1978). The pathogen is seldom severe enough to warrant fungicide applications in temperate climates (Gulya et al. 1997). However, the development of resistant hybrids becomes economically important if the crop is to expand into warmer regions where the disease may cause economic losses. Annual *H. debilis* ssp. *silvestris*, *H. praecox* ssp. *praecox*, and *H. bolanderi*, and 14 perennial species were tolerant of powdery mildew in both field and greenhouse tests (Saliman et al. 1982). Not all populations of perennial species are resistant; populations of *H. grosseserratus* and *H. maximiliani* showed differential reactions. Škorić (1984) reported that interspecific hybrids with *H. giganteus*, *H. hirsutus*, *H. divaricatus*, and *H. salicifolius* had no powdery mildew symptoms. Thirty-six accessions of Jerusalem artichoke (*H. tuberosus*) were field-screened to determine their reaction to powdery mildew, with three accessions showing high levels of tolerance (McCarter 1993). Christov (2008) identified perennials *H. decapetalus*, *H. laevigatus*, *H. glaucophyllus*, and *H. ciliaris* as sources of powdery mildew resistance. He indicated that there are two types of resistance to this pathogen. The first is controlled by a dominant gene from *H. decapetalus*. The second is controlled by multiple genes found in *H. glaucophyllus*, *H. ciliaris*, *H. laevigatus*, *H. debilis*, *H. tuberosus*, and *H. resinosus* possessing such resistance. Jan and Chandler (1985) identified a source of resistance to powdery mildew in *H. debilis* ssp. *debilis* that is incompletely dominant in the F_1 and backcross progenies. They incorporated genes from this species into a cultivated background and released a germplasm having the PM1 gene (Jan and Chandler 1988). Rojas-Barros et al. (2004) reported that two subspecies of *H. debilis* ssp. *debilis*, *H. debilis* ssp. *vestitus*, and *H. argophyllus* were completely resistant. The two *H. debilis* sources were different than those identified by Jan and Chandler (1985). Rojas-Barros et al. (2005) reported that patterns of segregation for powdery mildew resistance in *H. argophyllus* and *H. debilis* confirm the heterozygosity of the wild parent. Segregation patterns indicated that resistance in both crosses was controlled by at least two genes.

Rhizopus

Rhizopus head rot, caused by *Rhizopus* species, is an important sunflower disease in arid regions. It has become an important disease of sunflower in the USA (Rogers et al. 1978). The disease reduces oil quality and quantity in oilseed sunflower (Thompson and Rogers 1980). Infection of sunflower with *Rhizopus* head rot is enhanced by larval feeding of the sunflower moth, *Homoeosoma electellum* (Hulst), which contributes to a secondary infection (Rogers et al. 1978). The *Rhizopus* pathogen complex consists

of three species, with *Rhizopus arrhizus* Fischer the more prevalent and virulent species. Currently, cultivated sunflower does not possess resistance to *Rhizopus* head rot. Yang et al. (1980) reported that four out of 32 wild species and subspecies were resistant when inoculated with *R. arrhizus* and *R. oryzae* Went. The resistant sources were perennial *H. divaricatus*, *H. hirsutus*, *H.* x *laetiflorus*, and *H. resinosus*. Further breeding will be needed to transfer the identified sources of resistance into cultivated sunflower and to determine the inheritance of the resistance (Yang 1981).

Charcoal Rot

Charcoal rot, caused by *Macrophomina phaseolina* (Tassi) Goid, attacks sunflower and other crops in warm climates on all continents. Interspecific hybrids based on *H. tuberosus* have resistance to charcoal rot. Wild species *H. mollis*, *H. maximiliani*, *H. resinosus*, *H. tuberosus*, and *H. pauciflorus* have also shown resistance. Resistance to charcoal rot is positively correlated with resistance to Phomopsis stem canker and Phoma black stem (Škorić 1985). The inheritance of resistance to the pathogen has not been ascertained, or the number of genes involved, although resistance appears to be dominant.

Sunflower stem weevil can be a vector in transmitting the pathogen to sunflower. Yang et al. (1983) indicated that a small percentage of adult sunflower stem weevils carry *M. phaseolina* externally and transmit the pathogen to sunflower during oviposition via the sealed egg cavity in the stalk.

Broomrap parasite

Broomrape, caused by *Orobanche cumana* Wallr., is a parasitic weed that infects sunflower roots causing severe crop losses in Southern Europe and the Black Sea region (Höniges et al. 2008). It has also been observed in Australia, Mongolia, and China and is generally associated with drier climates. Five resistance genes (Or_1 through Or_5) have been used successfully for broomrape control following the progression of races A through E. Since broomrape is a highly variable parasite, the breakdown of resistance is a frequent phenomenon, and multiple sources of resistance are needed. The sources of resistance to *Orobanche* races found in the early sunflower breeding programs in the Former Soviet Union (FSU) originated from land races of cultivated sunflower, but genetic resistance was also introduced into susceptible sunflower from wild species, mainly *H. tuberosus* (Pustovoit et al. 1976). The early FSU cultivars and *H. tuberosus* were also important sources of resistance for the broomrape complex of races in Romania (Vrânceanu et al. 1980). Several investigators (Fernández-Martínez et al. 2000, 2008; Nikolova et al. 2000; Bervillé 2002) reported that sunflower germplasm evaluations for resistance to broomrape races have demonstrated that the *Helianthus* species constitute a major reservoir of genes conferring resistance to new virulence races.

A new broomrape race in Spain, designated race F, capable of overcoming all previously effective resistance genes was identified by Dominguez et al. (1996). High levels of resistance to races E and F have been found in the wild *Helianthus* species by Ruso et al. (1996) and Fernández-Martínez et al. (2000). They found resistance to races E and F in 29 perennial wild species, while very low levels were found in annual species, with only four of eight species evaluated showing some resistance to race F. Ruso et al. (1996) evaluated wild annual and perennial sunflower species' reactions to Spanish races and found two annual species, *H. anomalus* and *H. exilis*, that had resistance, and all 26 perennial species tested were resistant. Crossing perennial species with cultivated sunflower can be difficult, but with the use of embryo culture and chromosome doubling of the F_1s, amphiploids that facilitate the transfer of broomrape-resistant genes from the wild perennial species can be created. Using these techniques, amphiploids of perennial wild species *H. grosseserratus*, *H. maximiliani*, and *H. divaricatus* were produced that were resistant to race F (Jan and Fernández-Martínez 2002) and led to the release of four germplasm populations resistant to race F, named BR1 through BR4 (Jan et al. 2002). Resistance to race F appears to be controlled by dominant-recessive epistasis, complicating the breeding by requiring the genes to be incorporated into both parental lines of a resistant hybrid (Akhtouch et al. 2002). Pérez-Vich et al. (2002) studied the inheritance of resistance to race F derived from interspecific amphiploids with *H. annuus* and with two wild perennials, *H. divaricatus* and *H. grosseserratus*. They suggested that the resistance is controlled by a single dominant gene. In a re-examination by Velasco et al. (2006), however, the resistance of the sunflower germplasm J1 derived from *H. grosseserratus* proved to be digenic, the second gene being influenced by environmental factors.

Interspecific hybrids based on *H. eggertii* and *H. smithii* showed total resistance to broomrape in Bulgaria (Christov et al. 1998). Broomrape resistance to the local race in Bulgaria was reported in *H. divaricatus*, *H. eggertii*, *H. giganteus*, *H. grosseserratus*, *H. glaucophyllus*, *H. mollis*, *H. nuttallii*, *H. pauciflorus* (=*rigidus*), *H. resinosus*, and *H. tuberosus* (Christov 1996a). Also in Bulgaria, resistance to broomrape (race not specified) was reported in different progenies of interspecific hybrids with *H.*

pumilus by Nikolova et al. (2004). Diploid perennial species *H. divaricatus*, *H. giganteus*, *H. glaucophyllus*, *H. grosseserratus*, *H. mollis*, *H. nuttallii*, and *H. smithii* and their interspecific hybrids were resistant to broomrape (Nikolova et al. 1998). Christov (2008) reported that several perennial *Helianthus* species showed 100% resistance including *H. tuberosus*, *H. eggertii*, *H. smithii*, *H. pauciflorus*, and *H. strumosus*. Christov (2008) concluded that resistance to broomrape found in cultivated sunflower in Bulgaria is controlled by one dominant gene.

The interaction between *Orobanche* and the roots of wild sunflowers has been studied by Labrousse et al. (2001). Roots of an interspecific hybrid derived from *Helianthus debilis* ssp. *debilis* produced an impassable encapsulation layer that blocked the intruding parasite, which then died. Another interspecific hybrid from the same species showed reduced stimulation of broomrape seed germination and rapid necrosis at an early stage of parasite development. Resistance also occurred in an interspecific hybrid derived from *H. argophyllus* occurring mainly at stage four of the parasite development with no broomrape seed production observed because necrosis occurred before the broomrape flowered.

Resistance to broomrape has been observed in most of the wild perennial species (Ruso et al. 1996; Fernández-Martínez et al. 2000). Early reports of broomrape resistance were from the FSU where cultivars 'Progress' and 'Novinka' were developed using the "Group Immunity" breeding approach (Pustovoit and Gubin 1974). Immunity to broomrape in lines derived from *H. tuberosus* was also described by Pogorietsky and Geshle (1976). Škorić (1988) and Christov et al. (1996 a, b) concluded that the wild *Helianthus* species constitute the major reservoir of genes controlling resistance to the new virulent races of broomrape.

CONCLUSIONS AND PROSPECTS

The challenge for the sunflower breeding community is to breed sunflower adaptable to marginal environments and at the same time increase seed yield. Sunflower cultivation continues to be pushed into lower-fertility soils and other marginal environments where drought and high or low temperatures continually take their toll on the yield per unit area.

Sunflower hybrids have an extremely narrow genetic base, using only a single cytoplasmic male sterile source for all hybrids, making the crop extremely vulnerable to an impending disaster as seen in maize in the 1970s. There remains a need to increase the genetic diversity of cultivated sunflower due to the marked reduction in genetic diversity during domestication. Wild species of sunflower have been a source of resistance to many pests, especially for diseases. They also provide the cytoplasm that is the basis of all hybrid sunflowers. Since wild sunflower and the sunflower crop are native to North America, associated pests have coevolved in natural communities, thus providing the opportunity to search for pest resistance genes in the diverse wild species. Significant progress has been made in collecting and preserving wild species, increasing the genetic diversity available for crop improvement. Thus far, only a small portion of the available diversity has been exploited.

Significant advances have been made in understanding the origin, domestication, and organization of the genetic diversity, characterization, and screening methods for abiotic and biotic stresses in sunflower. The future direction will include the transfer of target genes from wild relatives into domesticated sunflower better adapted to local conditions. This will be facilitated by introgression of favorable alleles from alien germplasm, pyramiding favorable alleles and QTLs for specific disease traits, and simultaneously improving multiple traits. New sunflower hybrids will possess disease resistance genes from distantly related or even unrelated plants and other organisms. To keep sunflower an economically viable global crop, researchers must strive to combine the best conventional and modern molecular approaches available. This will require a multidisciplinary team approach and a commitment to a long-term integrated genetic improvement program.

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I. RESULTS OF SUNFLOWER BREEDING ON RESISTANCE TO DISEASES

Reproductive function potential of broomrape parasitizing on sunflower in the Rostov region

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ABSTRACT

The broomrape (*Orobanche cumana* Wallr.) morphotypes not described earlier are presented, which were found out in its most virulent populations in the Rostov region. The ability of this broomrape species is shown at parasitizing on sunflower to form plural adventitious shoots from tuber tissues, apical and basal parts of rudimentary roots and in axils of stem scales. The structures similar somatic embryos are found out on a surface of tuber basal part. The role of the specified changes in increase of reproductive function potential of *O. cumana* is discussed.

Key words: sunflower, broomrape, adventitious shoots, tuber, inflorescence, reproductive function

INTRODUCTION

The malicious weed broomrape (*Orobanche cumana* Wallr.) is an obligate parasite from among the higher floral herbaceous plants; it belongs to the family Orobanchaceae of the Scrophulariales order. As it has no roots, leaves and chlorophyll, this form of broomrape parasitizes on sunflower. In the conditions of the high degree of sunflower fields infestation broomrape can destroy the whole yield.

During the centenary history of sunflower cultivation in Russia there have been three periods when heavy affection of sunflower fields by broomrape threatened disappearance of sunflower. The joint evolution of parasite and host has led to the development of new broomrape races able to overcome the immunity of resistant sunflower varieties and hybrids.

In early 1970s varieties and hybrids resistant to the widespread by then Moldavian race of broomrape were developed. Their extensive cultivation has led to the annihilation of the broad plant-parasite seeds reserves in soil. Problem with broomrape on sunflower in Russia has not arisen right up to the beginning of this century. However, in recent years from different places of the Stavropol, Krasnodar, Rostov and Volgograd regions, there has been received information about affection of earlier resistant sunflower assortment by broomrape and strong infestation of sunflower fields in some areas (Antonova et al., 2009a). In the course of recent researches (Antonova et al., 2009b) new virulent broomrape races were differentiated in different districts of the Rostov region. Diversity of morphological and some other traits of parasitic plants were identified.

The purpose of this research is a description of the diversity of broomrape morphotypes from its most virulent populations in districts of the Rostov region.

MATERIALS AND METHODS

Survey was made on sunflower fields of the Tatsinsky, Konstantinovsky and Belokalitwinsky districts of the Rostov region at the broomrape seeds ripening stage. A collection of parasitic plants differing by morphotypes was gathered. Seeds of parasite from the examined fields were also gathered and used for creation of the infectious background in the open ground prepared at the central experimental base of VNIIMK. Observations of the parasite development on this area were conducted in 2009 during the whole period of sunflower vegetation by the digging out parasite and host plants and washing roots. Early stages of broomrape plants development (tubercle formation and other) on sunflower roots were observed with the help of stereoscopic microscope after washing 30-days host plants grown in sand-soil compound with broomrape seeds from the specified districts under the greenhouse conditions.

RESULTS AND DISCUSSION

It is well-known that *O. cumana* is a polyphage species. Being mainly the sunflower parasite, it can be also detected on tobacco, rustic tobacco, tomatoes, safflower, perilla, hemp, zinnia, kok-saghyz, tau-saghyz, on Artemisia maritima, Artemisia austriaca, Artemisia absinthium, Artemisia vulgaris, xanthium, wild lettuce, sea aster, mayweed (Kott, 1959; Baylin, 1968). Also a successful development of parasite on roots of abutilon and dandelion was observed.

D.T. Kabulov (1961) noted that obvious differences of morphological traits are peculiar to *O. cumana* by the development on different species of host plants. Clearly defined lability of morphological traits of polyphage species reflects their ability to adapt to new, often heterogeneous host plants species (Teryokhin, Ivanova, 1965).

Our observations showed that at present the diversity of morphological traits of this species can be detected even on the same host – sunflower. So, on one of the fields of the OJSC "Zazerskoye" (Tatsinsky district of the Rostov region) there were stems of broomrape 90 cm high (fig. 1 b) by its average rate of 40 cm (fig. 1 a) on plants of sunflower hybrid Makhaon. Besides, the overground part of the plant was an inflorescence. Seed capsules in axils of all leaf scales on the overground part of plant can be seen on fig. 1 b. Flowering inflorescence of broomrape with presence of flowers down to the ground level is shown on fig. 1 c. Such inflorescences were observed also on the infected plot of the central experimental base of VNIIMK where broomrape from the districts of the Rostov region, including Tatsinsky district, was applied. Stems of 93 cm high were described for this species in the last century in Bulgaria (Stoyanova et al., 1977), but the phenomenon of inflorescence being the whole overground part of the plant haven't occurred in the literature earlier. In "Flora of the USSR" it is stated that spike (spike-like inflorescence) of *O. cumana* on sunflower is generally crumbly, equal to the rest part of the stem or longer than it, has far disposed lower flowers (Novopokrovsky, Tsvelyov, 1958). Typical *O. cumana* inflorescence is shown on the fig. 1 a. We consider increase in the number of flowers and, accordingly, seed capsules on one stem as a way of amplification of the reproductive function of *O. cumana* in the course of its evolution on sunflower.

The first stage of description in the determination of Orobanchaceae along with other traits includes the following: "simple stems or stems growing right from the base as a bundle" (Beck-Mannagetta, 1930; Teryokhin, 1988). It is well-known that type of vegetative reproduction in weed populations of *O. cernua* (syn. *O. cumana*) parasitizing on the sunflower in Europe is a reduced form of "the perennial haustorial root type" with a very low potential for the reproduction of shoots from the tubercle tissues (Teryokhin, Chubarov, 1996). As it was noted by these authors, vegetative reproduction of this species on sunflower is limited by the formation of 1-3 shoots from the tubercle. In the meantime, tubercle is being transformed into the base of a main stem or stems with a corolla of rudimentary roots.

In the course of our research it was found out that along with single stems (fig. 1 a) there were many samples with 2-3 or 4-6 stems from one tuber (fig. 2, fig. 4 c, d) in the examined populations. Moreover, there were observed also multi-stem samples (fig. 3, fig. 4 a, b, fig. 5) with 10-40 stems from one tuber. Such form of *O. cumana* on sunflower has not been described earlier.

Quite often additional shoots were formed from the reduced roots of broomrape tuber (fig. 6). We observed new shoots emerging from the basal and lateral parts of tubercle, as well as from apexes of rudimentary roots. Thus, in the course of the joint evolution with sunflower rudiments of *O. cumana* roots have acquired reproductive function.

Phenomenon of formation of multiple adventitious shoots and reproductive function of reduced roots have not been observed before on broomrape parasitizing on sunflower. They were described for *O. cernua* parasitizing on tobacco in India by E.S. Teryokhin and S.I. Chubarov (1996).

We also refer formation of adventitious buds in tissues of new shoots to the broomrape phenomena not described previously. Additional shoots could develop in axils of leaf scales both of tuber and main shoot stem (fig. 7). Generally, damaged or affected stem was forming a few new shoots from the axils of leaf scales in addition.

Also undifferentiated structures from the meristematic cells similar to somatic embryos located on the basal surface of tuber have been observed (fig. 6). We suppose that epidermal cells of *O. cumana* tuber have acquired ability of spontaneous somatic embryogenesis. Such type of the Orobanchaceae reproduction has not been described in the literature. It reveals limitless opportunities for keeping of genotype and species in total, its evolution in the conditions of constant annihilation.

It seems that emergence of new adventitious shoots of some broomrape samples on sunflower in the observed populations has transformed into the continuous process during the whole vegetation period. Some samples have a rather interesting peculiarity: while the main stem was at the ripening stage, great

number of other were at the different stages of development and the last, the weakest ones were forming buds and tended to flower even before reaching the soil surface (fig. 8).

It should be noted that stems' bases both of main and adventitious broomrape shoots were notable for hypertrophied sizes and were comparable to the thickness of sunflower stem (fig. 7). It indicates their strongly increased storage function, what enables all new additional shoots to form fruits and seeds.

Areas of sunflower roots affected by broomrape are often ugly swollen (fig. 9, 10). However, such phenomenon is variable. Binding areas without swelling have been also found. A question occurs: is swelling of sunflower roots the consequence of influence of broomrape biotypes notable for the intensified storage function? For the present it is impossible to answer this question unambiguously, because examined weed populations can be described as a mixture of races both avirulent and particularly virulent to the cultivated sunflower assortment. This question demands special study, although it is logical to assume that some weed biotypes have developed ability of intensified spread of haustorial tissues within the host root in order to get firmer connection with root and nutrition of tuber with increased weight.

The morphogenesis of the examined broomrape populations, which was described above, indicates the evolution of parasite towards the intensification of reproductive function. Of itself it not only promotes its fast dissemination, but also leads to the faster development of new physiologic races and explains the phenomenon of their wide spreading. It should be also noted that broomrape tubercle has undergone maximal evolutional changes, what indicates its greatest role in the ontogenesis of a parasite.

In the Rostov region a decrease of fields in crop rotations and frequent return of sunflower to the previous place in 1-3 years are being observed. This has led to the development of new aggressive biotypes of broomrape, which have overcome the immunity of the resistant sunflower assortment and spread quickly over wide areas. In accordance with the new knowledge about the high potential of broomrape reproductive function the cultivating treatment of rows on sunflower fields used by farmers of the region is considered as absolutely inadmissible. In such a case the overground parts of broomrape plants are destroyed, and their rests in sunflower roots realize their potential of reproductive function by forming new adventitious shoots in large quantities.

Thus, one of the distinctive peculiarities of broomrape from the examined populations is the increasing of the storage function of tuber and stems' bases, expressed in the hypertrophied enlargement of these structures.

Another distinctive peculiarity of sunflower broomrape is an extremely high potential of reproductive function. Increase of number of seeds can take place due to the formation of flowers and fruits with seeds in axils of all scales (reduced leaves). Besides, during the vegetation period broomrape from the examined populations is able to form multiple adventitious shoots developing in tissues both of an initial tuber and rudimentary roots, as well as in axils of leaf scales of main and emerged shoots. On the surface of the basal part of tubercle there have been discovered undifferentiated meristematic structures similar to somatic embryos.

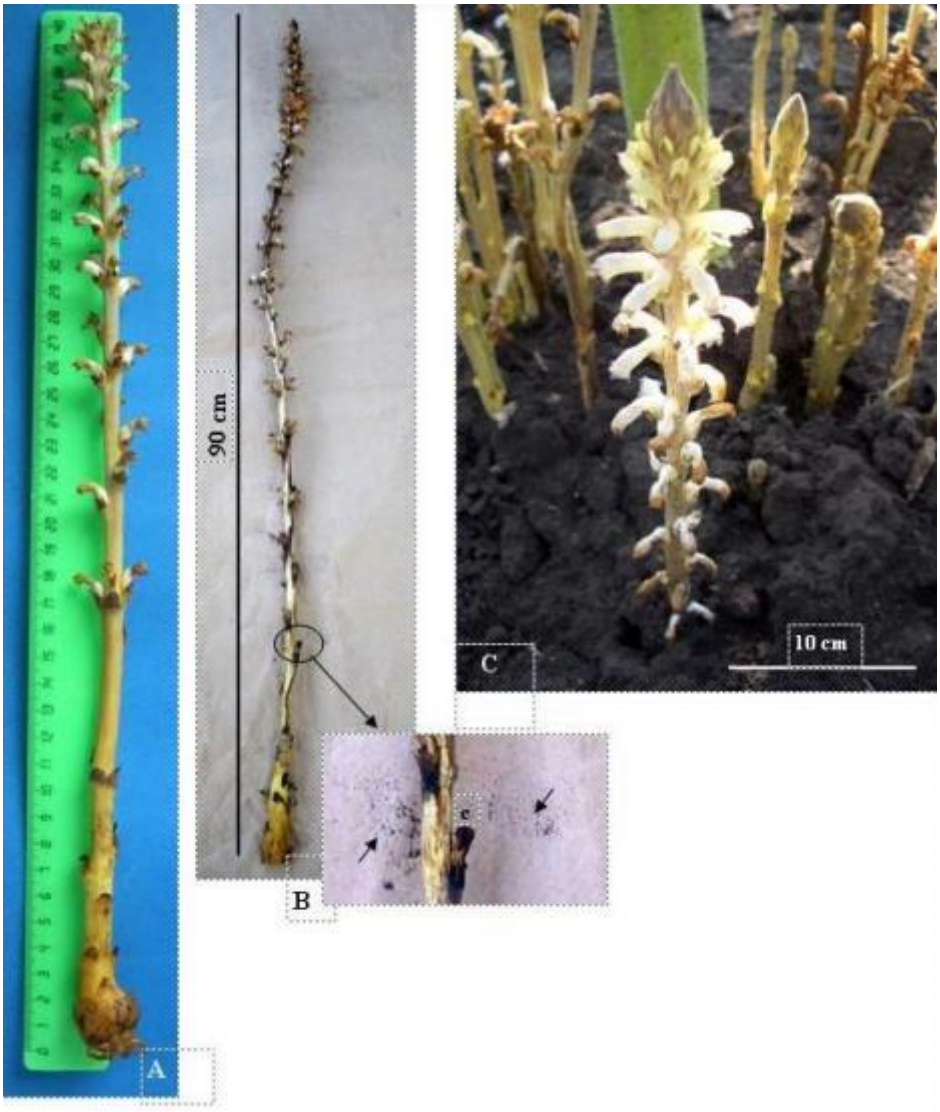

Fig. 1. *Orobanche cumana* Wallr. plant: A - normal type; B, C - stem is an inflorescence as a whole: (B) - after the finish of flowering, plant 90 cm long; ripe fruits - capsules (C) with seeds are observed (pointed with arrows); (C) - flowering one; flowers develop straight from the soil surface level.

Fig. 2. Forms of *Orobanche cumana* Wallr. with 2-4 stems from the very virulent populations of the Rostov region.

Fig. 3. Multi-stem form of *Orobanche cumana* Wallr. from the very virulent populations of the Rostov region.

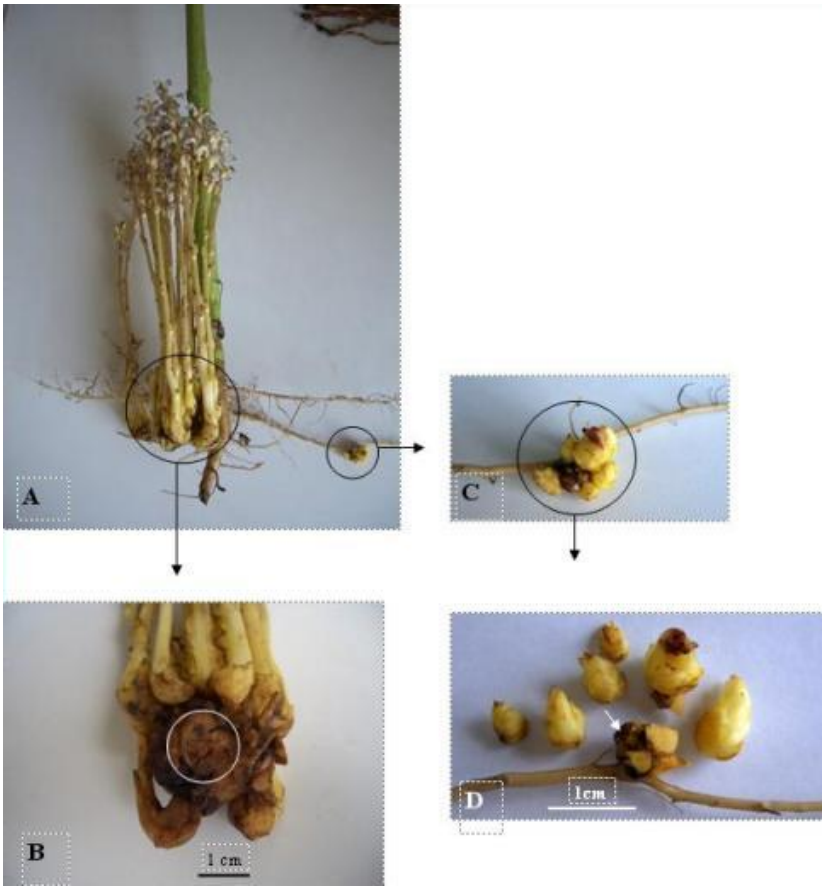

Fig. 4. A - washed sunflower roots with a flowering multi-stem sample of *Orobanche cumana* Wallr.;

B - broomrape tubercle common for all stems, view from its basal part; encircled is the area of connection with sunflower root;

C - Sunflower root with one tubercle and six sprouts on it;

D - Sprouts separated from the tubercle (pointed with arrow).

Fig. 5. Development of adventitious shoots from one tuber of broomrape *Orobanche cumana* Wallr. parasitizing on sunflower: A - overall view, MR - main root of sunflower; B - view from the basal part of tuber (T) branching from the sunflower root, C - areas of connection between tuber and root.

Fig. 6. Adventitious shoots (AS) of broomrape developed from the reduced roots of the tuber (T) and five structures (encircled) similar to somatic embryos (SE) developed on the surface of its basal part.

Fig. 7. Stem of broomrape (B) with a tuberous base and adventitious shoots (pointed with arrows) branching both from the tuberous base and from leaf scale axils of the stem; SR - main root of sunflower.

Fig. 8. Multi-stem form of broomrape *Orobanche cumana* Wallr. with flowers of two adventitious shoots which exploded under the ground surface (pointed with arrows): T - tubercle; MS - main flower-bearing stem.

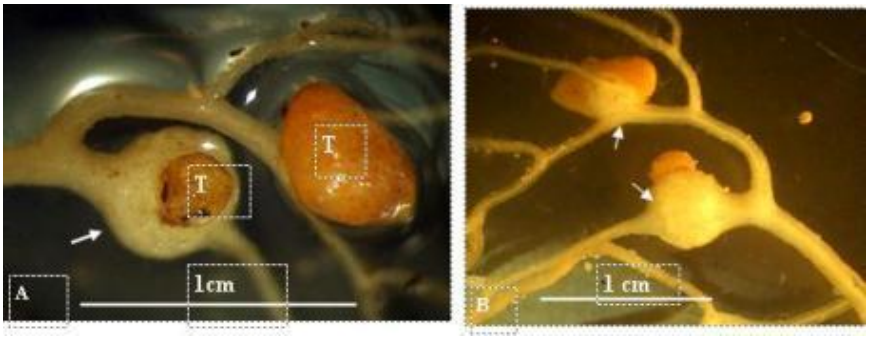

Fig. 9. Sunflower root swellings (pointed with arrows) around the haustorium of broomrape *O. cumana* at the early stages of tubercle (T) development: A - from above, B – from the lower side of roots and tubercles.

Fig. 10. Deformed swellings on roots of sunflower plant affected by broomrape. Areas of connection between sunflower roots and broomrape stems are pointed with arrows; B - stem of the ripe broomrape branching from the swollen root; SR - the main root of sunflower.

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Screening and developing new sunflower hybrids for resistance to Alternaria, Powdery Mildew and Leaf Crinkle virus in Uganda

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ABSTRACT

Sunflower (*Helianthus annuus L*) has become an important oil crop in Uganda especially in the eastern and northern districts of the country. By late 1980, Uganda was importing 98% of the total edible oil in the country. Considering the high oil content compared to other oilseeds, sunflower shows the greatest potential in reducing Uganda's dependence on imported edible oil. Despite the importance of sunflower now in the country, there are challenges in sunflower production in Uganda. Among the challenges are diseases. The main diseases affecting sunflower in Uganda are Alternaria, Sclerotinia (root, stem and head rot), Powdery mildew, and Leaf crinkle virus disease.

Sunflower genotypes were evaluated at the National Semi Arid Resources Research Institute (NASARRI) and at Ngetta Zonal Agricultural Research and Development Insttitute (NgeZARDI) in 2008 and 2009 growing season under rainfed conditions. The main objective was to screen for genotypes with resistance or moderate resistance to the main sunflower diseases that reduce yield in this country. The genotypes were planted in randomized complete block design with three replications. The spacing was at 75 x 30 cm leaving one plant per hole after thinning. Data recorded were the rate of attack by the main diseases such as Alternaria, leaf blotch and crinkling, powdery mildew and seed yield per plot, head diameter. The scale used for scoring diseases were 1-9 where 1 is very resistant and 9 is highly susceptible to that disease. Results showed that there were differences in the attack of the different genotypes by these main diseases of sunflower in Uganda. Particular lines are being selected to be used in developing new hybrids for this country.

Key words: disease tolerance – hybrid development – screening

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is becoming the main oilseed crop in Uganda especially in the eastern and northern districts of the country. Its oil is used mainly as cooking oil and for soap making while the seed cake is being used as livestock feed. Vegetable Oil Development Project (VODP) impact assessment report (VODP, 2007) reported that the total acreage of sunflower production in Uganda by 2006 was 298,642 ha with an output of 358,320 MT of seed.

Uganda showed interest in sunflower production in 1947 (Bua and Molo, 1985). Research in sunflower that was begun in 1970s and 1980s did not show any impact in sunflower production in Uganda because the government by then did not put much interest in its research development. Research done was mainly on direct evaluation of imported hybrids and open pollinated varieties of the Russian varieties. These did not reach the commercial production stage as no variety was officially released. Other factors included low price in the market, low adoption of agronomic practices and there were less than 4 oil mills known. The crop therefore remained an ornamental crop grown around homesteads.

By 1990's, Uganda still was importing more than 95% of the cooking oil. A few oil mills were then installed in places like Lira and Gulu districts. By 1995, the only sunflower variety available was the local stripe open pollinated variety that was grown in the North and was possibly imported by Missionaries and possessed only 29% oil content. Then there was the open pollinated sunflower variety 'Sunfola' that was officially released in 1991. It was found to be possessing high oil content of over 46% and a softer seed coat that could easily be crushed without abrasing the machines.

As sunflower is now being popularized in Uganda, a number of diseases and pests have been recorded. The main diseases affecting sunflower production in Uganda are: white rot (*Sclerotinia sclerotiorum),* Alternaria blight (*Alternaria helianthi*), Sunflower viral leaf blotch and leaf crinkle. Of late powdery mildew (*Erysiphe cichoracearum* DC.ex Meret) has shown to be prevailing during maturity period although its symptom was less seen in the past. For pests, the main ones are birds (*Quelea quelea*, weaver birds, doves), insects such as *Helicorverpa armigera* (bollworm) is the main destructive insect on sunflower followed by grasshoppers and semi loopers sporadically. Sunflower is normally grown in Uganda during the second rainy season which is from July to December. Due to availability of market, it

is now also being grown in the first rainy season which is between March to June which is normally for food crops.

Several viruses and virus like diseases have been identified by various workers on sunflower around the world. This includes: Sunflower mosaic, yellow mosaic, chlorotic mosaic, yellow ring mosaic, yellow mosaic, yellow spot, cucumber mosaic, curl mosaic and Mycoplasma like organisms. In Uganda, some leaf sample attacked by the virus was sent to Kenya and Britain for identification but no conclusive proof of the specific virus was reported. Theuri *et al*.1987 recorded that sunflower Yellow Blotch and Leaf Crinkle (Luteovirus) are two different symptoms which are caused by closely related viruses in several African countries including Kenya, Malawi, Tanzania and Uganda. Yellow Blotch disease is characterized by bright-yellow blotches on the leaves, but plant height and leave morphology are not affected. Some plants show more severe symptoms after yellow blotch appearance. The youngest leaves are twisted, reduced in size and often curled downwards. While leaf crinkle disease symptoms consist of irregular yellow vein banding as with yellow Blotch diseases but also include severe leaf puckering, starting with the youngest leaves, reduced leaf size and stunted growth. The leaf crinkle disease is similar to that described earlier as rugose mosaic (Gulya *et al*. 1997). Both viral diseases are mechanically transmissible, and the aphid *Aphis gossypii* was able to vector Yellow Blotch disease. (Gulya *et al*. 1997). It is also reported that there is no known sources of resistance for these viral diseases. Therefore, no breeding programme has been designed to incorporate resistance to this virus except cultural methods such as removing weeds to destroy the virus source and avoid the primary inoculums to the crop, destruction of the infected plants as soon as they are noticed to avoid further spread in the field. No insecticidal sprays are used to control insects in sunflower in Uganda. No source of resistance has been mentioned elsewhere.

Importance of resistance breeding

To control the diseases, cultural methods have been recommended. A chemical method used has been only on seed dressing by seed companies. However, use of chemicals is not only cost prohibitive but causes environmental hazards. Application of fungicides may result in pesticide resistance due to strong selective pressure on the pathogen. Developing disease resistant varieties/hybrids is therefore essential to bring down the cost of production besides being friendly. With climatic change causing drought and floods, resistance breeding is the best choice.

This paper therefore presents the results of screening for the main sunflower diseases in Uganda and how breeding for resistance against them can be developed.

MATERIALS AND METHODS

During the second rainy season (July-Decemeber) 2008, twenty seven genotypes were evaluated across locations at the trial verification centres of Serere, Ngetta and Kumi . During the first rainy season (March-June) 2009, twenty five genotypes were evaluated. and in the second rainy season of 2009, thirteen genotypes were evaluated only at Serere. Each plot had four rows and two middle rows were used for data recording. The spacing was 75 x 30 cm at a length of 4 m long. Three replications were used in the experiments. Plants were thinned to one plant at seedling stage. No fertilizer was applied since most farmers do not use fertilizer in their fields. Yield data was obtained from the two middle rows during harvest. Disease scores were rated at a scale of 1 to 9 for Alternaria, leaf blotch and crinkle virus disease, powdery mildew during physiological maturity. In some cases percentage of attack for leaf blotch and crinkle virus disease was undertaken.

RESULTS AND DISCUSSION

In Table 1, at Serere research institute during second rainy season in 2008, most genotypes had moderate resistance to Alternaria recorded at a scale of 3. Hybrids from crosses with restorer R274 and R694 had high incidence of leaf blotch and leaf crinkle disease compared to the rest of other genotypes. This could be seen in 371A/R694, 372A/R694, 404A/R694 and 432A/R694 scoring a scale of 5 and 6. It was only in 372A/R274 that had low incidence of leaf blotch and leaf crinkle. Where leaf blotch and leaf crinkle attack at physiological maturity, yield loss is negligible.

In Table 2, at Ngetta Zonal research institute during the second rainy season in 2008, there was less incidence of leaf blotch and crinkle for all the genotypes but high incidence of Alternaria disease. Incidentally, crosses to restorer R694 which are late maturing, had less incidence of Alternaria attack. These are seen in 371A/R694, 383A/R694 and 432A/R694 recording scale of 3. Although they scored

high rate for resistance, there yields were low. No significant difference was recorded in the incidence of leaf crinkle in all the genotypes.

In table 3, at Serere research institute during the first season of 2009, all the genotypes had high incidence of Alternaria disease. PAN 7034 which had the least Alternaria incidence at 40% also had the highest yield of 1750 kgha . Genotypes with low Alternaria incidence also recorded had high seed yield. Significant difference was recorded among genotypes for leaf blotch and leaf crinkle virus disease. Eleven genotypes had score of less than 3 indicating that they are tolerant to the disease. There was no significant difference among genotypes for powdery mildew incidence. Powdery mildew normally appears during physiological maturity. This disease has not been a problem but of late, its prevalence is increasing. Hegde (Personal communication) also expressed the recent prevalence of powdery mildew in India.

In Table 4, at Serere research institute during the second rainy season of 2009, there was generally high incidence of Alternaria on all the genotypes. Better genotypes were 383A/R271, PAN 7033, PAN 7369 recording scale of 4. The incidence was high because during this season, the rainfall was high through out the growing season which is conducive for the prevalence of Alternaria.. Leaf blotch and crinkle incidence were low and non significant. Chattopadhyay (1999) in India also recorded that Alternaria disease is important in humid areas and yield loss may range from 15 to 90% with 20-30% oil loss.

In other countries, sunflower has been broadened by the infusion of genes from the wild species. Diseases limit production in a majority of sunflower producing countries. Sunflower is a host to a wide number of diseases that can cause serious economic damage in terms of yield and quality. There are reports of identification of cultivated sunflower genotypes with low susceptibility or moderate resistance to Sclerotinia white mold. Wild species have been identified as potential source of genes for Sclerotinia tolerance such as H. maximiliani, H.nuttalli, H. resinosus, H.tuberosus, H.mollis.

Morris *et al*. 1983, recorded that all 21 annual taxa and 18 of 21 perennial species of wild sunflower evaluated were susceptible to A.helianthi (Hansf.) Tub. And Nish. Perennial species such as *H.hirsutus, H. pauciflorus* subsp. Subrhomboideus and *H. tuberosus* appear to resist infection by *Alternaria helianthi.* Lipps and Herr (1986) showed that 13 accessions of *H. tuberosus* had significantly less Alternaria leaf spot than commercial hybrids and concluded that the species is a potential source of resistance to leaf spot. Others are *H. praecox, H. x laetiflorus* Pers; *H.debilis* subsp. Cucumerifolius, and *H. debilis* subsp. Silvestris with high levels of resistance to Alternaria.

Table 1. Evaluation of sunflower genotypes at Serere during the season of 2008B

	Genotype	Yield kg/ha	Vigour	Maturity	Alternaria	Leaf crinkle
1	371A/R271	1306	3.3	95	3	4
2	371A/R274	1139	4.0	92	3	5
3	371A/R373	1400	2.7	92	3	5
4	371A/R694	1305	4.0	93	4	6
5	372A/R271	1111	5.0	97	3	5
6	372A/R274	1222	4.3	93	3	3
7	372A/R373	1389	3.3	93	3	5
8	372A/R694	1500	4.0	95	3	6
9	383A/R271	1334	2.7	92	3	4
10	383A/R274	1167	2.0	89	3	5
11	383A/R373	1548	3.0	91	3	4
12	383A/R694	1194	2.7	93	3	5
13	404A/R271	1556	2.3	91	3	4
14	404A/R274	1083	3.7	92	3	6
15	404A/R373	1306	3.3	92	3	5
16	404A/R694	1028	4.0	92	4	6
17	432A/R271	1222	4.3	91	3	6
18	432A/R274	1306	3.7	90	3	5
19	432A/R373	1361	3.0	91	3	6
20	432A/R694	944	3.3	93	3	5
21	PAN7033	1833	4.0	94	3	5
22	PAN7034	1583	3.7	93	3	3
23	PAN7351	1445	4.3	94	3	6
24	PAN7369	1584	3.3	94	3	3
25	PAN7371	889	3.3	95	3	3
26	Sunfola	1278	4.0	91	4	3
27	Record	1139	3.3	93	4	3
	Mean	1303	3.5	93	3	4.6
	C.V%	22	24	3.0	17.5	44
	LSD	ns	1.38	NS	0.94	NS
	Prob level (5%)	0.071	0.017	0.401	0.041	0.64

Scale:
Vigour: 1= Very vigorous 9= Least vigorous
Alternaria: 1= Immune 9= Very susceptible
Leaf blotch and crinkle: 1= Immune 9= very susceptible

Table 2. Evaluation of sunflower genotypes at Ngetta during the season of 2008B

	Genotype	Yield kg/ha	maturity	Alternaria	Leaf crinkle virus
1	371A/R271	833	92	4.7	0.7
2	371A/R274	556	85	4.7	0.3
3	371A/R373	722	91	4.0	1.3
4	371A/R694	611	95	3.0	0.0
5	372A/R271	1000	90	5.0	1.3
6	372A/R274	722	90	4.7	0.3
7	372A/R373	1000	91	4.7	0.0
8	372A/R694	1166	100	4.0	1.0
9	383A/R271	889	90	4.7	0.0
10	383A/R274	556	82	5.7	1.0
11	383A/R373	778	91	4.3	1.3
12	383A/R694	389	98	3.0	0.0
13	404A/R271	611	88	5.0	0.0
14	404A/R274	389	85	6.0	0.7
15	404A/R373	222	88	4.7	1.0
16	404A/R694	250	91	5.5	0.1
17	432A/R271	833	85	5.3	0.3
18	432A/R274	389	84	5.3	0.3
19	432A/R373	611	88	5.0	0.3
20	432A/R694	722	90	3.0	0.3
21	PAN7033	1944	95	4.0	0.3
22	PAN7034	1278	93	4.7	0.0
23	PAN7351	1333	93	5.3	1.3
24	PAN7369	833	97	5.0	0.0
25	PAN7371	1111	91	4.3	0.0
26	Sunfola	444	86	6.7	0.0
27	Record	628	96	5.3	0.0
	Mean	771	91	4.7	0.44
	c.v%	29	2.4	12	
	lsd	368	3.6	0.92	NS
	Prob (5%)	0.001	0.001	0.001	

Table 3. Evaluation of sunflower genotypes at Serere during the season of 2009A

	Genotype	Yield kg/ha	Alternaria diseases %	Leaf crinkle disease	Powdery mildew
1	371/R271	417	55	2.7	3.0
2	371/R274	695	65	2.3	3.7
3	371/R373	833	58	3.0	3.0
4	371/R694	639	66	3.0	3.3
5	372/R271	806	70	3.0	3.3
6	372/R274	1222	42	3.7	3.0
7	372/R373	806	62	2.3	2.0
8	372/R694	500	59	2.7	2.7
9	383/R271	1444	62	2.7	2.7
10	383/R274	639	72	3.3	3.0
11	383/R373	778	59	2.7	2.3
12	383/R694	694	61	3.0	2.3
13	404/R274	583	63	3.7	3.0
14	404/R373	556	65	3.3	3.3
15	404/R694	417	77	3.7	3.0
16	432/R271	945	52	4.0	3.0
17	432/R274	555	62	2.7	2.7
18	432/R373	833	56	3.0	2.7
19	432/R694	833	59	2.3	2.7
20	PAN7033	1667	75	2.7	2.0
21	PAN7034	1750	40	2.0	2.7
22	PAN7369	1278	49	2.3	3.0
23	Sunfola	889	55	3.3	2.7
24	Record Tanzania	972	49	3.3	2.3
	Mean	865	60	2.9	2.8
	C.V%	47.6	23.8	20.6	24
	LSD (5%)	676	NS	0.99	NS
	Prob. Level (5%)	0.007	0.258	0.014	0.37

Table 4. Evaluation of sunflower genotypes at Serere during the season of 2009B

	Genotype	Yield kg/ha	Maturity	Alternaria disease (scale)	Leaf crinkle virus disease
1	371A/R271	792	94	4.5	1.0
2	371A/R274	875	90	4.5	1.5
3	371A/R373	500	92	5.0	1.0
4	372A/R271	916	94	5.0	0.5
5	372A/R373	791	91	5.0	2.0
6	383A/R271	1208	91	4.0	2.5
7	383A/R373	1042	91	4.5	2.5
8	PAN7033	1292	95	4.0	0.5
9	PAN7034	1542	93	4.5	3.5
10	PAN7351	1042	94	5.0	3.0
11	PAN7369	750	95	4.0	1.5
12	Sunfola	667	90	6.0	1.0
13	Record Tanzania	750	95	4.5	1.0
	Mean	936	93	4.7	1.7
	C.V%	29.5	1.7	9.1	74
	LSD	NS	3.3	0.9	NS
	Prob. Level (5%)	0.104	0.037	0.021	0.37

CONCLUSION

Healthy plants are essential for good crop performance. Identification of genes for resistance to plant disease pathogen and incorporation of genes for resistance to diseases into cultivars improves plant performance and reduces the need for the chemical disease control (Poehlman and Sleper, 1995). Breeding for insect resistance is an economical and an environmentally sound means for avoiding insect damage while reducing the use of pest control chemicals in field and horticultural crops.

In order to breed for disease and pest resistance, there is need to develop methodology for conducting effective and efficient screening methods that can show good results. The use of wild sunflower species will be very important in crossing with the cultivated sunflower. Exchange of germplasm resistant or tolerant to some of these diseases and pests will help to develop better varieties with resistant genes. Knowledge on mode of disease and pest resistance is very important in breeding for resistance. Vear (2004) expressed that although there have been 40 years (4 decades) of research on sunflower genetics, this is still young compared to other crops like wheat, barley or potato.

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Evaluation of sunflower parental forms and its hybrids for damage caused by Phomopsis pathogen under artificial infection background

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ABSTRACT

The results of evaluation for damage breeding lines and hybrids of sunflower caused by phomopsis pathogen during 2007-2009 ys under artificial infectious background are stated. The method of differentiation of materials for sunflower stem surface area affected by phomopsis pathogen according to the confidence interval of LSD is used. From the-3-year data of phytopatological evaluation of pathogen affection the hybrids possessing better characteristic every year - HC 26 A / Kh 785 R, SKh 2111 A / Kh 526 R, SKh 2111 A / Kh 843 R, SKh 2122 A / Kh 843 R are selected. Kh 526 R, Kh 720 R, Kh 480 R are also selected as resistant among the paternal lines and SKh 1010 A, SKh 908 A, SKh 4021 A, SKh 2111 A - among maternal ones that is stipulated for certain by the least level of their damage with phomopsis pathogen.

Key words: pathogen – hybrid – infectious background – line – sunflower – affection – resistance – Phomopsis

INTRODUCTION

Resistance of cultivated plants to the effect of biotic factors in the combination with a high potential productivity is a main condition for the provision of a high stable yield of agricultural crops. In this connection in adaptive selection a particular place is given to the study of variation and inheritance of corresponding adaptive reactions, the integrity of which is genetically determined and stipulated by multiple ties at a level of both separate plants and biocenosis at large. The main factors of plant and hybrids with high potential productivity, high plant densities, the application of high rates of nitrogen fertilizers in most cases reduce the resistance of agrocenosis to the effect of biotic factors. Just these circumstances move forward the task to increase potential yield of cultural plants. which are prominent for their high resistance to unfavorable environmental conditions [1].

The basic method of selection for resistance is hybridization with the use of disease-resistant forms. Its success depends on the validity of phytopatological estimation [2].

MATERIALS AND METHODS

In 2007-2009 ys 39 hybrids of F_1, as well as, their paternal forms: 5 lines – restorers of pollen fertility as paternal forms and 9 – maternal lines were tested for resistance to phomopsis under artificial infectious background.

Fields experiments with sunflower were conducted on the isolated phytopatological site on 0,5 ha area in the 3-years monoculture conditions. Planting data was 2 weeks later than recommended one for the north-eastern part of the Forest-Steppe of Ukraine.

The infectious background was created according to our synthesized methods of the Russian [3], Serbian [4] and French [5] scientists. The average weighed index of disease's severity [6] was calculated according to A.E. Chumakov's method [7]. The differentiation as to affection degree of the sunflower experimental material was carried out according to severity scoring scale with provision for the statistical criterion - LSD [8]. Generalizations of researches results were made by means of the standard packet of data analysis of Microsoft Excel (licensed № XJT36-B8T7W-9C3FV-9C9Y8-MJ226).

RESULTS AND DUSCUSSION

The weathers conditions in the vegetation period of sunflower during 2007-2009 ys, were unfavorable for phomopsis pathogene's development. Hydrothermal coefficient (HTC) brought confirmation of the statement and which was calculated during the crop vegetation. So, moisture level of sunflower sowing during each of three years studies was 0,7 (0,68 - 0,71), that is considered as drought. Generally, such conditions as those limit the break of diseases of a necrotrophic type, in particular, of phomopsis, in sunflower sowings. However, with equal values of HTC the infection level was changed. Under close consideration of HTC for each vegetation month in sunflower it is obvious that HTC of June -

1,51 affected hybrids in 2007 (fig. 1). Infection level rise in 2009 was possibly stipulated by HTC value growth up to 0,85 in July. The infection level of phomopsis this year is notable for the highest value - 22,4 % of the affected area of plants stems during the years of researches.

When studying HTC effect in each months of vegetation period of sunflower on the level of pathogen development by correlation analysis method a direct positive connection is observed between the infection level and hydrothermal conditions of July (r = 0,68...0,76).

The least level of disease manifestation on infectious background under irrigation is observed in 2008 when revealing varietysamples without the symptoms of affection by causal agent of phomopsis.

So the minimum index of affection was 0,3 % of disease intensity. In 2007 that index was 1,3%, in 2009 - 4,25 %, on the average - 5,93 % of the stem area affected by phomopsis causal agent (fig. 1).

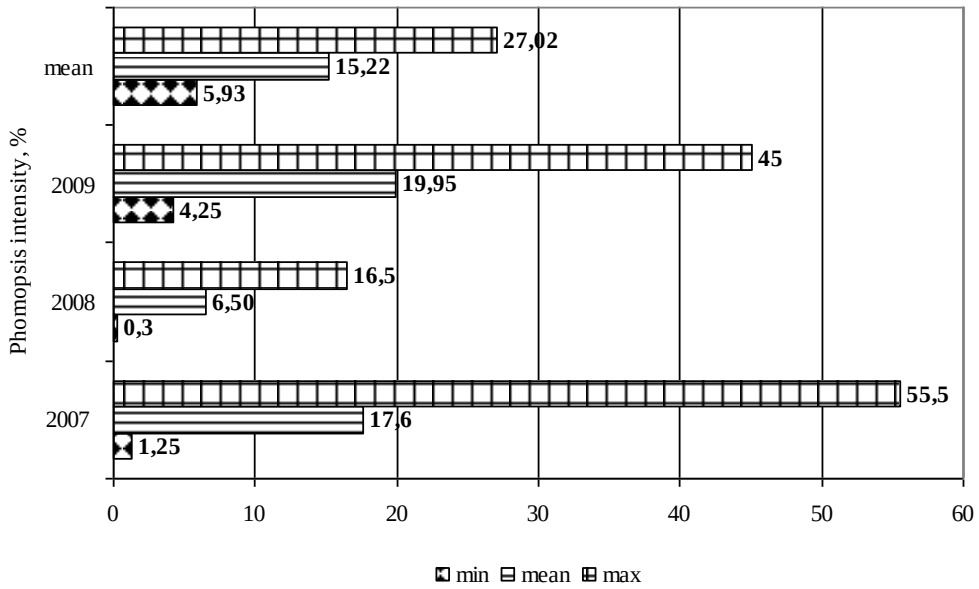

Fig. 1. Phomopsis intensity variation in sunflower hybrids under artificial infection, 2007-2009 ys.

The mean value of phomopsis by the sum total of hybrids of sunflower in the years of investigations of disease intensity was: 17,6 % in 2007, 16,5 % in 2008, 19,9 % in 2009 and middle for 2007-2009 - 15,22 % of stem area affected by the phomopsis pathogen.

The maximal values of disease level were high in 2007 and 2009. The three year mean value is defined as intermediate (27,02 %), between the values of previous years and in 2008 (6,5 % of stem area affected by disease pathogen).

The maximum index of hybrids' affection had the highest value in 2007. However, a high level of mean value of affection by the sum total of hybrids in 2009 shows also the larger number, than in 2007. Therefore, the conditions in 2009 for certain were more severe for the estimation of resistance of sunflower genotypes to phomopsis causal agent.

In connection with a wide spread of phomopsis under contrasting intensity, as for years, of the disease development the hybrids and their paternal forms, in correspondence with the reaction to the damage caused by phomopsis pathogen, up divided into the groups in accord to LSD criterion [3, 20].

Table 1 shows statistically defined limits of sunflower hybrids by the intensity of disease development. In 2007 the least affected hybrids had from 1,3 to 13,4 % of stem area affected by disease pathogen. Hybrids with mean degree of affection - from 13,5 % to 21,8 %, but those with maximum level - from 21,9 to 55,5% of disease intensity.

The grouping of hybrids conducted in accord with the intensity of infection in 2008 possessed such a level of affection: the minimum affected hybrids - from 0,0 to 4,4 %; averagely affected - from 4,5 % to 6,9 %; maximum affected - from 7,0 to 16,5 % of stem area.

Table 1. Differentiation of sunflower hybrids for the level of affection caused by phomopsis pathogen in accord with the confidence interval parameters - LSD (2007-2009 ys)

Group	Group limits as to affected stem area, (mean ±LSD), %			
	2007	2008	2009	mean
Minimum affection	1,3…13,4	0,0…4,4	4,3…17,1	5,9…13,5
Mean affection	13,5…21,8	4,5…6,9	17,2…22,9	13,6…16,9
Maximum affection	21,9…55,5	7,0…16,5	23,0…45,0	17,0…27,0
Mean for experiment	17,6	5,7	19,9	15,2
$LSD_{0,05}$	4,2	1,3	2,9	1,7

In 2009 to the group of the hybrids with minimum affection were attributed those, which showed from 4,3 to 7,1 % of stem area damaged by disease pathogen; to the group of mean affected - from 17,2 % to 22,9 % and to the maximum affected - from 23,0 to 45,0 %.

When averaging the three-year data the group limits are characterized as such: minimum affected hybrids - from 5,9 to 13,5 % of stem area colonized by a pathogen; hybrids with mean affected - from 13,6 % to 16,9 %; those with maximum damage - from 17,0 to 27,0 %.

The groups contain the hybrids being combined by a definite level of the trait investigated. The definite differences were yearly revealed in percent ratio within the groups (Fig. 2).

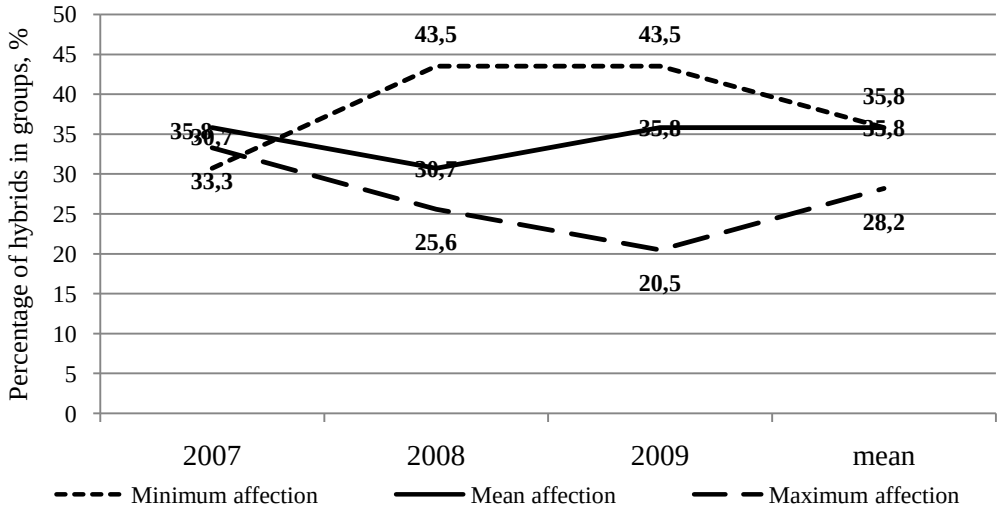

Fig. 2. Percentage of hybrids within the groups by the level of affection caused by phomopsis pathogen in the years of investigations (2007-2009 ys)

When describing the variation in the number of hybrids within the group with minimum affection it can be seen that the largest and equal part of hybrids, particularly, 43,5 % was attributed to it in 2008 and 2009 years. In 2007 the least of all hybrids - 30,7 % entered that group.

The group of midaffected hybrids is more variable. In 2009 it was the least (20,5 %), the intermediate group was in 2008 (25,6 %). The percent ratio in the group of midaffected hybrids in 2007 was approximately similar to that with midaffected hybrids as to the averaged three-year data, 28,2 % and 30,7 %, respectively.

The group of the most affected hybrids was equally distributed in the years: 35,8 % of samples in 2007, in 2009 and averaged three-year data, and 30,7 % hybrids in 2008.

The detailed characteristic of 9 hybrids of sunflower, selected for minimum affection as to the data of phytopatological estimation under artificial infectious background is given in Table 2.

Table 2. Sunflower hybrids selected for the least level of affection caused by phomopsis pathogen under artificial infection background, 2007-2009 ys.

Nos.	Hybrid combination	Phomopsis intensity, %			
		2007	2008	2009	mean
1	HC 26 A / Kh 1228 R	7,0*	4,3*	26,3	12,5*
2	HC 26 A / Kh 785 R	7,0*	1,0*	14,3*	7,4*
3	SKh 1008 A / Kh 1228 R	23,5	1,5*	11,8*	12,3*
4	SKh 1008 A / Kh 526 R	7,0*	8,3	14,5*	9,9*
5	SKh 1010 A / Kh 526 R	1,3*	14,0	16,3*	10,5*
6	SKh 1012 A / Kh 526 R	2,5*	9,5	16,5*	9,5*
7	SKh 2111 A / Kh 526 R	4,8*	1,5*	11,5*	5,9*
8	SKh 2111 A / Kh 843 R	4,8*	4,0*	11,8*	6,9*
9	SKh 2122 A / Kh 843 R	10,9*	1,8*	13,8*	8,8*
	Mean for experiment	17,6	6,4	20,0	15,2
	$LSD_{0,05}$	4,2	1,3	2,9	1,7

From the sum total of hybrids 5 with significantly low level of affection as to mean 2-year data were identified - SKh 1012 A / Kh 526 R, SKh 1010 A / Kh 526 R, SKh 1008 A / Kh 526 R, SKh 1008 A / Kh 1228 R, HC 26 A / Kh 1228 R. With regard to mean 3-year data 4 hybrids were selected: - HC 26 A / Kh 785 R, SKh 2111 A / Kh 526 R, SKh 2111 A / Kh 843 R, SKh 2122 A / Kh 843 R. At this point the latter had the best characteristic every year.

The individual characteristic of the components of crossing - sterile maternal lines and lines-restorers of pollen fertility of sunflower was studied as well.

In Table 3 the results of phytopathological evaluation of lines and their differentiation in the groups for level of infection.

Table 3. Differentiation of lines of sunflower groups for level of infection caused by phomopsis pathogen (2007-2009 ys)

Lines	Stem area infected, %	Immunological evaluation
SKh 1010 A, SKh 908 A, SKh 4021 A, Kh 720 R, Kh 480 R, Kh 526 R, SKh 2111 A	2,8* - 11,4*	Least infected
SKh 1012 A, CH-43 A, SKh 1002 A, SKh 2552 A, SKh 1008 A, Kh 785 R	13,9 – 15,1	Mean infected
SKh 1006 A, SKh 2122 A, HC 26 A, Kh 1228 R, Kh 843 R	18,6 – 32,0	Most infected
Mean for experiment	15,2	
$LSD_{0,05}$	1,7	

As to the maternal lines SKh 1010 A showed a minimum level of disease infection during 2-year studies (2,8 %).

The lines of SKh 908 A and SKh 4021 A are also referred to the group of the least affected ones as to average value of stem area affected by phomopsis pathogen (5,0 - 5,3 %). The maternal line SKh 2111 A had the highest affection (11,4 %) among the genotypes with minimum affection. Among the lines-restorer of pollen fertility of sunflower Kh 720 R, Kh 480 R, Kh 526 R with the values of 5,3 - 8,0 % of stem area infected by the pathogen are included in this group.

To the mid-infected group as to the values of 13,9 -15,1 % the maternal lines of SKh 1012 A, CH-43 A, SKh 1002 A, SKh 2552 A, SKh 1008 A and paternal line R 785 R are included.

The maternal lines SKh 1006 A, SKh 2122 A, HC 26 A and restorers Kh 1228 R and Kh 843 R with the values of 18,6 - 32,0 % of stem area infected by the pathogen are referred to the maximum affected group.

Thus, under artificial infections background the differentiation of sunflower hybrids in the groups is carried out in accord with LSD statistical parameter, which has been estimated by stem surface area affected by phomopsis pathogen. In connection with the data of 3-year studies (2007-2009 ys) the hybrids of HC 26 A / Kh

785 R, SKh 2111 A / Kh 526 R, SKh 2111 A / Kh 843 R, SKh 2122 A / Kh 843 R are selected with significantly low infection, which had the best characteristic every year. The lines Kh 526 R, Kh 720 R, Kh 480 R, as resistant among the paternal forms are identified and SKh 1010 A, SKh 908 A, SKh 4021 A, SKh 2111 A - among the maternal ones that is stipulated by significantly their low affection by phomopsis pathogen.

The use of intensity index of phomopsis development in sunflower, which is estimated by stem surface area of affection, permits to generalize the data from causal agent development in sunflower hybrids under low or contrasting levels of disease severity.

When calculating HTC in each months of sunflower vegetation it has been revealed that the hydrothermal conditions of July, in particular, at flowering stage had an influence on the level of affection of hybrids in the conditions of north-eastern part of Forest-Steppe.

Established peculiarities will allow to increase the efficiency of plant-breeding work in the future study of the lines obtained from 3-year data of investigations, and the hybrids involved in the study have a great practical value – they are being studied as for breeding traits in the programs at the laboratory for breeding and genetics of sunflower of the Institute.

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Creating new sunflower forms and lines, resistant to diseases, the broomrape parasite and certain types of herbicides applying the interspecific and intergeneric hybridization

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ABSTRACT

The achievement of a potential for high productivity in the new modern sunflower hybrids under field conditions depends directly on both the method of breeding and the sunflower lines resistant to widespread diseases, the broomrape parasite, and certain types of herbicides, used for the creation of hybrids. Therefore, we have included in the study interspecific and intergeneric hybrids, obtained by crossing cultivated sunflower with annual and perennial species of the *Helianthus* and other genera of the *Compositae* family, which have demonstrated resistance to diseases of economical importance and the broomrape parasite. We have applied purposeful selection and self pollination; crossing of individual interspecific and intergeneric hybrids and self pollination in the next generations; crossing with lines for evaluation of the general and the specific combining abilities and productivity, evaluation of the resistance to diseases, broomrape parasite and the Pulsar and Express herbicides, evaluation of the presence of *Rf* genes, oil contents in the seeds, and some other interesting features and properties. The result of the research study is the obtaining of a large number of forms and lines /B and R/ with full resistance to the diseases downy mildew, phomopsis, phoma, alternaria, high resistance to sclerotinia, and full resistance to broomrape. We have established three new sources for resistance to the Pulsar herbicide.

Key words: broomrape – diseases – interspecific and intergeneric hybrids – herbicides – resistance.

INTRODUCTION

The achievement of a potential for high productivity in the new modern sunflower hybrids under field conditions depends directly on both the method of breeding and initial material - parental lines, their productive potential and resistance to wide spread diseases, broomrape parasite and some herbicides. Expression of higher productive potential and yield stability were achieved as a result of complete combination of increasing the genetic potential for resistance to diseases, pests, broomrape parasite, herbicides and soil and air drying up.

Sackston /1981/ pointed, that number of pathogens in the world exceeded 80 species for the period 1921-1961. It was typical for sunflower agrocenose that increasing of new pathogen species was observed /Mihailova, 1988/. Except new pathogens, the new races also occurred, which overcome genes resistant to the old races. Especially such problem occurred with the new races of downy mildew and broomrape. Fast increasing of plots under sunflower at the beginning of 80s the wide spread of new races was observed - 3, 4, 6 and 7 races in North America, races 7 and 3 in Argentina, races 3, 4 and 6 in Europe /Tourvieille et al., 1988; Gulya et al., 1991 and etc./. Seven or eight races were established for the broomrape.

In Bulgaria 40 pathogens were registered, but those with economical importance were extremely less. Diseases with serious influence on sunflower were downy mildew, sclerotinia wilt, phomopsis, broomrape parasite and with less influence - phoma, alternaria, charcoal rot. In some regions of the country, attacks of rhyzopus head rot and botrytis head rot were observed sometimes and much more restricted occurrence of verticillium wilt and powdery mildew.

Diseases and pests overcoming in sunflower were carried out in a genetic way. First attempts implemented by Satziperov /1916/ and connected to hybridization between *H. annuus* and *H. argophyllus* aimed to create immune forms with higher seed oil content. Many forms resistant to diseases and the broomrape parasite were obtained from crosses between cultivated sunflower *Helianthus annuus* L. and other wild species from genus *Helianthus* /Putt and Sackston, 1957, 1963; Pustovoit, 1960, 1966, 1975a; Pustovoit, 1963, 1967, 1969a, 1969b, 1973, 1975b; Leclercq et al., 1970; Cvetkova, 1976; Cuk, 1982; Skoric, 1985, 1987, 1988, 1992; Christov, 1990, 2002, 2006, 2008a, 2008b; Dozet et al., 1990; Seiler,

1992; Christov et al., 1996, 2004, 2009a, 2009b; Fernandez-Martinez and Ruso,1997; Bachvarova, 2004; Hristova-Cherbadzi, 2007 and etc./. Gulya et al. /1997/, Vranceanu /2000/ and Vear /2004/ summarized the collected information and established that greater part of the wild species and their varieties were carriers of genes for different diseases, pests and parasites. Transfer of gene plasma into the cultivated sunflower by applying the interspecific hybridization resulted in creation of many new forms for heterosis breeding.

New material resistant to some herbicides was developed /Al-Khatib et al., 1998; Bruniard, 2001; Fabie and Miller, 2002; Miller and Al-Khatib, 2002; Kolkman et al., 2004; Christov et al., 2008 and etc./.

In 1957 in Bulgaria a new cultivar № 2-19 was registered as resistant to broomrape, and developed by applying interspecific hybridization /Stojanova et al., 1977/. Good results in developing new forms and lines resistant to downy mildew and broomrape were obtained during the period 1972-1979. From 1983 till now the work on using of interspecific hybridization for developing initial material resistant to different diseases, pests, parasites and other stress factors for the needs of sunflower heterosis breeding was spread. The created collection was enriched with a large group of species of genus *Helianthus* together with native and foreign sunflower populations. All accessions in the collection were studied for resistance to main diseases and broomrape parasite spread on the territory of the country. Purposeful hybridization was carried out between cultivated sunflower and wild sunflower accessions possessed some resistance. In this investigation were included 34 species from genus *Helianthus* with more than 250 accessions, 16 cultivars and 18 cultivated sunflower lines. From 1985 till 1989, 32 species from family *Compositae* closely related and distant relatives of genus *Helianthus* were included. The aim was to develop F_1 interspecific and intergeneric hybrids, which had to possess genes controlling the resistance to some diseases and the parasite broomrape. As a result a hybrid material second and third generation was obtained, which could be base for developing lines with normal cytoplasm without *Rf* genes /B lines/ and lines with *Rf* genes /R lines/.

The aim of this report is to present our work on developing new sunflower forms and lines, resistant to diseases, the broomrape parasite and some herbicides using interspecific and intergeneric hybridization and to present some results of our investigations.

MATERIALS AND METHODS

The investigations were carried out during the period 1983 - 2009 on the territory of Dobroudja Agricultural Institute, Bulgaria.

Plant material

The plant hybrid material from F_1 to 26 hybrid generations was used. It was obtained from crosses between cultivated sunflower, presented by16 cultivars and 18 lines and 32 wild species: *Helianthus annuus* (w.f.), *H. argophyllus*, *H. bolanderi*, *H. debilis*, *H. neglectus*, *H. petiolaris*, *H. praecox*, *H. paradoxus*, *H. divaricatus*, *H. giganteus*, *H. glaucophyllus*, *H. grosseserratus*, *H. maximiliani*, *H. mollis*, *H. nuttallii*, *H. occidentalis*, *H. pumilus*, *H. salicifolius*, *H. smithii*, *H. decapetalus*, *H. hirsutus*, *H. laevigatus*, *H. scaberimus*, *H. tomentosus*, *H. eggertii*, *H. pauciflorus* /*rigidus*/, *H. resinosus*, *H. strumosus*, *H. tuberosus*, *H. ciliaris*, *H.* x *laetiflorus*, *H. californicus* from genus *Helianthus* and 26 other species from 22 genera - *Arctium*, *Aster*, *Bidens*, *Calendula*, *Carlina*, *Carduus*, *Carthamus*, *Cihorium*, *Ehinacea*, *Evmolpia*, *Gaillardia*, *Grindelia*, *Inula*, *Matrikaria*, *Onopordum*, *Silphium*, *Silybum*, *Telekia*, *Tithonia*, *Verbesina*, *Zinnia* and *Xanthium* from family *Compositae*.

Breeding methods

As a result of repeated interspecific and intergeneric hybridization more than 65 000 F_1 hybrid plants from interspecific and more than 1 900 F_1 hybrid plants from intergeneric hybridization were obtained. Obtaining of following generation was carried out by applying of different ways of pollination of hybrid plants. The repeated, purposeful and consecutive selection aimed the alignment the plants along the long period of time from one and the same accession in morphological, biological and biochemical traits. The selection broadened in time and in progress generations. In the first and usually in second and third generation it was limited because of the small number of obtained plants. It was done on the base of phonological observations, biometrical measurements and laboratory testing results. The hybrid material was studied for resistance to diseases, parasites, pests, drying and during the last 7-8 years for resistance to herbicides. The evaluation for seed oil content, ratio hull/kernel, 1000 seeds weight, seed set, general and specific combining ability, productive potential of the newly obtained lines and their hybrids was done.

Evaluation for resistance to diseases and the broomrape parasite was made of species and intergeneric hybrids on affirmed in the institute methodic /Iliescu, 1955; Pancenko, 1975; Tourvieille et al., 1988; Christov, 1990, 1996a, b; Christov et al., 1992, 1996, 2004; Alonso, 1996; Pacureanu-Joita et al., 1998; Fernandez-Martinez et al., 2000; Encheva and Kiryakov, 2002; Shindrova, 2006a, 2006b/. Hybrid material was studied for resistance to the diseases downy mildew (*Plasmopara helianthi* Novot.), sclerotinia wilt (*Sclerotinia sclerotiorum* (Lib.) de Bary), phomopsis stem canker (*Phomopsis helianthi* Munt.-Cvet. et al.), phoma black stem (*Phoma macdonaldi* / *oleraceae* var. *helianthi* Munt.-Cvet. et al.), alternaria (*Alternaria helianthi* (Hansf.) Tubaki and Nishihara and *Al. zinniae* Pape), powdery mildew (*Erysiphe cichoracearum* D. C.) and charcoal rot (*Sclerotium bataticola* / Macrophomina phaseoli (Tassi) Goid) and the parasite broomrape (*Orobanche cumana* Wallr.) under field and laboratory conditions. At first plants from all accessions were studied, and after F_2 and BC_1 only on selected accessions. The part of F_1 plants was small. During the process of evaluation 4 different groups of hybrid materials were selected and grouped separately for purposeful selection for resistance to one disease - sclerotinia, downy mildew, phomopsis and parasite broomrape with duration of the investigation: 7-8 vegetation periods /years/. This investigation began from the third hybrid generation. Different downy mildew and broomrape races were used as well broomrape from different regions in Bulgaria, Romania, Ukraine, Russia, Moldova, Turkey and Spain. In the investigations were included materials with different sources of resistance - different wild species and native forms, interspecific and intergeneric hybrids and cultivars and lines from different countries for comparison.

For resistance to herbicides two groups were selected for searching the presence to herbicides Pulsar 40 (40 g/l imazamox) - IMI type and Granstar and Granstar express San (50 и 75 % active ingredient tribenuron). In phase second-third true leaves pair of sunflower the herbicide combination Pulsar 40 (40 g/l imazamox) in dose 120 ml/dka + Stomp 330 EK(330 g/l pendimetalin) in dose 230 ml/dka with a back hand sprayer SOLO 425 with 40 l/dka working solution /Christov et al., 2008/ were carried in. The dose for Express 50 SG was 4g/dka with 60 l/dka working solution.

Seed oil and protein content and amino acid content were evaluated on the affirmed in the institute methods /Rushkovskii, 1957; Stojanova and Ivanov, 1968; Ivanov et al., 1996/. Nuclear magnetic resonance apparatus was used for evaluation of seed oil content and analyzer Hitachi, L-8500 for amino acids.

RESULTS AND DISCUSSION

Obtaining and preparation of hybrid plants for evaluation of their resistance to diseases and broomrape parasite.

In comparison with intraspecific hybridization, during wide hybridization seeds were obtained on rare occasions. In some cases there were no plants from seeds of part of the crosses. Some plants died, others were sterile. Greater part of the hybrid plants, obtained from perennials and part of annuals formed polled but were not self-pollinated. With aim to obtain the follow generation despite self-pollination, we did controlled pollination in a group between pants from one and the same cross, between plants from different crosses and backcross with cultivated sunflower. All F_1 plants were branched and this gave the possibility to make different combinations of pollination and to leave some inflorescences for free pollination with pollen from the nursery, where hybrid plants and their parental forms of cultivated sunflower were grown. The sterile plants also were pollinated with pollen from plants from the same cross or other accession. Pollination with cultivated sunflower - line, cultivar or mixed pollen was also made. Obtaining of small number of hybrid plants restricted the investigation in the first generation. This resulted in the evaluation of the next generations. It was impossible one, two or three hybrid plants to be evaluated at once for their resistance to several diseases and the parasite broomrape. In most cases plant died because of one disease and it might possess a resistance to other disease. It was established that some species and more accurate accessions combined resistance to two or three diseases and some of them to the parasite broomrape. In some materials other useful characters were transferred too, as high combining ability, dry tolerance, existence of *Rf* genes, diversity in amino acid content of the seeds and etc. On the other hand some of the characters could be lost or could be found in very small number of plants. It's necessitative to investigate bigger number of plants or selection of germs. Combining field and laboratory investigations helped for falling away of part of the additional difficulties. In that case quarantine plot was effective. The sunflower agrocenose progress and acceleration of new races virulence needed searching for resistance among more different materials. Methods of artificial inoculation were also of great interest. When sufficient number of plants and quantity of seeds obtained study of reaction to several diseases and broomrape could be carried out in first generation /F_1 plants/. When we obtained insufficient number of seeds the investigations began from second or third generation when the number of plants and

germs were enough. Hybrid plants obtained from tetraploid, hexaploid and some of diploid perennial species were with perennial life cycle. From one F_1 perennial plant next year we obtained several F_1 plants from the sleeping buds of the roots. Such type of F_1 plants reproduction gave the opportunity for control and verification the resistance to diseases and to the parasite broomrape. Another way with better opportunities was multiplication of F_1 and in some cases F_2 and BC_1 plants applying tissue culture.

With greater priority were the investigations of resistance to downy mildew (*Plasmopara helianthi* Novot. / *Pl. halstedii* (Farl.) Berl. and de Toni), sclerotinia (*Sclerotinia sclerotiorum* (Lib.) de Bary), phomopsis (*Phomopsis helianthi*/ *Diaporthe helianthi* Munt.-Cvet. et al.) and the parasite broomrape (*Orobanche cumana* Wallr.).

Creating of new sunflower forms resistant to downy mildew (*Plasmopara helianthi* Novot.).

Downy mildew could cause full dying of plants. It takes first place on its economic importance for sunflower production in the USA, Canada and European countries /Sackston, 1992/. It's known for this disease the presence of many races and fast appearance of new ones more virulent, which overcome the resistance of widely spread in practice cultivars and hybrids /Tourvieille et al., 1988; Sackston et al., 1990; Gulya et al., 1991 and etc./. The resistance is controlled by one dominant gene, which is presented by more alternative forms. Tourvieille et al. /2000/ suggested unified nomenclature for the race composition of downy mildew, where 10 races existent in the world were pointed.

There are five races № 300, 330, 700, 721 and 731 established on the territory of Bulgaria and existed 2008-2009. Breeding purposes developing of resistant forms, lines and hybrids to the last four mentioned races. Only results obtained from the laboratory investigations were determined as reliable. Among the hybrid forms, obtained with participation of big group of *Helianthus* species and some species from different genera from family *Compositae* the full resistant to all races was established. Resistance to races № 300, 330 and 700 was established in hybrid forms, obtained in participation of species *Helianthus annuus* (w.f.), *H. argophyllus, H. bolanderi, H. debilis, H. neglectus, H. petiolaris, H. praecox, H. paradoxus, H. divaricatus, H. giganteus, H. glaucophyllus, H. grosseserratus, H. maximiliani, H. mollis, H. nuttallii, H. occidentalis, H. pumilus, H. salicifolius, H. smithii, H. decapetalus, H. hirsutus, H. laevigatus, H. scaberimus, H. tomentosus, H. eggertii, H. pauciflorus /rigidus/, H. resinosus, H. strumosus, H. tuberosus, H. ciliaris, H.* x *laetiflorus, H. californicus, Arctium lappa*, *Aster speciosa*, *Bidens tripartita*, *Carduus acanthoides*, *Evmolpia* sp., *Gaillardia hybrida*, *Grindelia speciosa*, *Inula helenium*, *Matrikaria hamomila*, *Onopordum acanthium*, *Silphium perfoliatum*, *Telekia speciosa*, *Tithonia rotundifolia*, *Verbesina alata*, *Verbesina helianthoides*, *Verbesina encelioides*. Resistance to other two races № 721 and 731 was established in hybrid forms, obtain in participation of species *H. divaricatus, H. hirsutus, H. pauciflorus /rigidus/, H. debilis* sp. *debilis, H. paradoxus, Inula helenium*, *Tithonia rotundifolia* and *Grindelia speciosa*. Resistance to the last two races was found in only 310 hybrid forms. More than 96 % of all reistant plants possessed *Rf* genes. Some of these materials possessed resistance to other diseases and broomrape parasite. Predominant parts of materials were advanced 20^{th} - 26^{th} generations, 2670 accessions. Most of them were complete R lines. Four of them were parental forms of 6 already registered hybrid cultivars - hybrids. Number of lines with normal cytoplasm /B lines/ resistant to downy mildew was 81. Part of them was resistant to broomrape.

Group of materials, resistant to the most virulent race of downy mildew was determined. The aim of the present work is transfer of this resistance to R lines with other interesting characters, obtained before the new races appearance.

In table 1 and 2 the complete R lines are presented, which are obtained by using of interspecific and intergeneric hybridization and resistant to downy mildew.

Table 1. Characterization of sunflower lines, resistant to downy mildew, race 700 and obtained by the methods of interspecific hybridization, harvest 2007.

Accession, Pedigree	Generation	Resistance to, %		Seed oil content, %
		downy mildew	broomrape	
PR-6/7 /c.s.* x *H.pauciflorus* 028/	24	100	100	50.68
PR-27/7 /c.s. x *H.tuberosus* 037/	23	100	100	46.41
PR-29/7 /c.s. x *H.tuberosus* 051/	18	100	100	44.27
PR-31/7 /c.s. x *H.pauciflorus* 002/	25	100	100	51.56
PR-45/7 /c.s. x *H.nuttallii* 173/	15	100	100	60.11
PR-47/7 /c.s. x *H.bolanderi* 009/	17	100	100	52.96
PR-55/7 /c.s. x *H. eggertii* 001/	24	100	100	52.34
PR-61/7 /c.s. x *H.pauciflorus* 094/	19	100	100	55.42
PR-57/8 /c.s. x *H.debilis* 011/	24	100	0	49.46
7009R /c.s. x *H.tuberosus* 037/	24	100	100	45.64
7043R /c.s. x / *H.pauciflorus* 002/	24	100	100	52.65
7203R /c.s. x *H.decapetalus* 043/	24	100	100	49.98
C-23 /c.s. x *H.debilis* 011/	23	100	100	47.12
C-27 /c.s. x *H. H.tuberosus* 037/	17	100	100	52.96
C-28 /c.s. x *H. divaricatus* 044/	24	100	100	52.34
C-37 /c.s. x *H.debilis* 104/	18	100	100	47.55
C-40 /c.s. x *H.praecox* 029/	23	100	100	50.30
6134B /c.s. x *H.debilis* 011/	22	100	100	48.18
6874B /c.s. x *H.decapetalus* 043/	24	100	100	48.56

*c.s. - cultivated sunflower

Table 2. Characterization of sunflower lines, obtained by interspecific and intergeneric hybridization, resistant to downy mildew-race 731, harvest 2009.

Accession, Pedigree	Generation	Resistance to, %		Seed oil content, %
		downy mildew	broomrape	
PR-1/8 /c.s. x *H.pauciflorus*/	23	100	100	48.48
PR-9/8 /c.s. x *H.tuberosus*/	25	100	100	47.27
PR-13/8 /c.s. x *H.pumilus*/	16	100	-	58.28
PR-25/8 /c.s. x *H.pauciflorus*/	25	100	100	46.89
PR-35/8 /c.s. x *H.hirsutus*/	16	100	100	48.80
PR-41/8 /c.s. x *H.divaricatus*/	18	100	100	47.03
PR-51/8 /c.s. x *C.acanthoides*/	18	100	-	52.96
PR-56/8 /c.s. x *Aster speciosa*/	17*	100	100	49.56
PR-57/8 /c.s. x *Inula*/ x *Tith*.	16	100	100	50.35

* - unbranched form

Creating new sunflower forms, resistant to sclerotinia (*Sclerotinia sclerotiorum* (Lib.) de Bary).

Sclerotinia wilt /*Sclerotinia sclerotiorum* (Lib.) de Bary/ is an extremely destructive fungus, which influence on the economic could carry the collapse for many crops and for sunflower /Fick and Miller, 1997/. The reasons for the great damages of sclerotinia are long term storage of pathogen in the soil and the great number of hosts. This pathogen attacks all parts of the stem. Sclerotinia attacks determine several forms of manifestation: root form, stem form - infection covers the middle stem part and leaves, infection of head and infection of seeds /Skoric, 1988/. The disease symptoms were various. They included wilt and basal stem rot, breaking of the mid-stem, head rot caused by ascospore infection and sometimes leaf and cotyledon infection. Damages could be about 100 %. Cultivated sunflower is high susceptible to *Sclerotinia sclerotiorum*.

Investigations on Sclerotinia resistance were carried out under field conditions and in nurseries. Different ways for artificial inoculations were used. Inoculation was carried out at one day and at one and the same growth stage of plants. Mycelium of fungus was used for inoculation. Sclerotia and ascospores were also used. First evaluations were made with mycelium put with big quantities only on the roots and covered with wet soil. After that the better methods were used. The isolate was grown on PDA tissue. The

inoculum was prepared in one closed end plastic straw /drinking straw/. The inoculation was carried out in phase button formation of sunflower. Leaf petiole of mid-stem leaves was cut on 20-25 mm from leaf base. The straw with inoculum was put on the cut surface and the fungus mycelium touched the plant tissue directly. The results were checked after 3, 7, 11, 14, and 30 days after inoculation on 6 levels scale - from 0 to 5 /Encheva and Kiryakov, 2002; Christov et al., 2004/. Inoculum was put on all parts of the sunflower plant - root, stem, branches, leaf petioles and inflorescences.

Wild species and interspecific and intergeneric hybrids from different generations were inoculated with mycelium and sclerotia. Ascospore infection was used only for plants in advanced generations.

Despite the total evaluation of hybrid material, group of 200 hybrid forms mainly obtained from intergeneric hybridization, was collected and evaluated consecutively 8 vegetation periods /years/ for resistance to Sclerotinia. Annually from each accession were collected plants which showed high level of resistance - from 0 to 2 and possessed good seed set. Mainly after 5th year or sometimes after 3rd year all plants from an accession showed uniformity regarding their level of resistance. Except one accession all others possessed *Rf* genes. Regarding their level of resistance there were three interesting groups. There were 37 accessions with grades 0-1, 49 accessions with grades 2 and 21 accessions with grades 2-3. There was a fourth group too where the accessions were included later. In the 4th group each plants grades were from 0 to 3. Sixteen accessions from groups with grades 0-1 and 2 were included in 2009 in 23 hybrid combinations. Experimental results for Sclerotinia resistance were presented in tables 3 and 5.

Table 3. Characteristic of R lines obteined from intergeneric hybrids as a result of purposeful research breeding for high resistance to Sclerotinia, harvest 2008.

Accession, Pedigree	Resistance to,			Seed oil content,
	sclerotinia, grades 0 - 5	downy mildew, %	broomrape, %	%
Sc-16 /c.s. x *Silfium* sp./	1	100	0	49.95
Sc-17 /c.s. x *Grindelia* sp./	0	100	0	49.11
Sc-20 /c.s. x *Mdtricaria* sp./	1	100	0	46.30
Sc-23 /c.s. x *Telekia* sp./	0	100	0	51.39
Sc-25 /c.s. x *Carduus* sp./	1	100	100	46.23
Sc-28 /c.s. x *Carduus* sp./	1	100	100	48.50
Sc-29 /c.s. x *Tithonia* sp./	1	100	100	45.28
Sc-38 /c.s. x *Inula* sp./ x Tith.	0	100	100	49.98
Sc-58 /c.s. x *Tithonia* sp./ x Verb.	2	100	0	48.12
Sc-61 /c.s. x *Zinnia* sp./	1	100	-	43.27
Sc-66 /c.s. x *Arctium* sp./	0	100	100	46.51
Sc-138 /c.s. x *Inula* sp./ x Tith.	2	100	80	43.78

Creating of new sunflower forms resistant to phomopsis (*Phomopsis helianthi* / *Diaporthe helianthi* Munt.-Cvet. et al.), phoma (*Phoma macdonaldi* / *oleraceae* var. *helianthi* Munt.-Cvet. et al.) and alternaria (*Alternaria helianthi* (Hansf.) Tubaki and Nishihara и *Al. zinniae* Pape).

Phomopsis /*Phomopsis helianthi* Munt.-Cvet. et al./ is one of the newer diseases on sunflower. It's first epidemic manifestation in Europe was registered in former Yugoslavia, district Voivodina on July 1980 /Mihaljčević et al., 1980, 1982; Skoric, 1985/. Disease spread very quickly in neighboring countries. In Bulgaria first symptoms of this disease were established in 1983. Now Phomopsis occurred in all regions in the world, where sunflower was grown. It attacks all above ground parts of the plant but damages on stems were biggest. Attacked tissues get soft and stem prone to lodging at the place of infection. Necrosis reached deeply in stem and fungus hypha reached the stem axis and neighboring tissues.

Investigations connected Phomopsis resistance began in 1987 in our institute when first evaluation of species was made under field conditions. First selection of plants from wild species from genus *Helianthus* showed certain resistance was made in 1989. First crosses were done then. First for evaluation was used natural infection in nursery with hybrid plants. Afer that the artificial infection was used. Now the evaluation was made on the method developed by Encheva and Kiryakov and published in 2002.

For the period 1989 - 2009 a great number of hybrid materials were developed and beside them there were plants which showed high level of resistance. They were the base for creating by selection 50 lines with high resistance to Phomopsis.

Together with the investigations for Phomopsis resistance, investigations on Phoma resistance began too (*Phoma helianthi* Munt.-Cvet. et al.). Rarely could be observed serious attacks of Phoma in sunflower. The lesions observed at the places where leaf petioles connected stem like black spots. Because of wilting the tissues began fragile. Infection maintained at our conditions in the quarantine plots. During the last years the evaluations were done by artificial inoculation of plants on the method of Encheva and Kiryakov /2002/.

Results of investigations on Phomopsis and Phoma resistance were presented at tables 4 and 5.

Table 4. Characterization of sunflower lines, obtained by interspecific and intergeneric hybridization, harvest 2007.

Accession, Pedigree	Generation	Resistance to, grades 0-4		Seed oil content, %
		Phomopsis	Phoma	
№ 1375 /c.s. x *Tithonia* sp./	19	1	0	43.75
№ 1386 /c.s. x *H. decapetalus*/	20	0	0	49.31
№ 1391 /c.s. x *Tithonia* sp./	18	1	0	50.82
№ 1400 /c.s. x *H. occidentalis*/	16	0	0	47.55
№ 1405 /c.s. x *Carduus* sp./	16	0	0	45.92
№ 1419 /c.s. x *Carduus* sp./	16	0	0	52.65
№ 1421 /c.s. x *H.pauciflorus*/	20	0	0	54.38
№ 1434 /c.s. x *H. paradoxus*/	19	1	0	50.70
№ 1460 /c.s. x *H.pauciflorus*/	20	0	0	49.24
№ 1612 /c.s. x *Tithonia* sp./	18	1	0	50.44
№ 1811 /c.s. x *H. ciliaris*/	19	1	0	48.05
№ 1816 /c.s. x *H. resinosus*/	19	1	0	47.82
№ 1850 /c.s. x *H. decapetalus*/	20	0	0	52.35

Alternaria (*Alternaria helianthi* (Hansf.) Tubaki and Nishihara и *Al. zinniae* Pape) attacks all above plant organs. It attacks germs, form dry spots on slems, leaves and inflorescences and reach seeds. It worsens quality of seeds and infects sowing material. Investigations on Alternaria resistance (*Alternaria helianthi* (Hansf.) Tubaki and Nishihara and *Al. zinniae* Pape) began later. Investigations in great detail were done during the period 1985-1989 of wild sunflower species. Then the first crosses for developing resistant forms were carried out. After that period only hybrid forms were studied. During the last years the work was carried out on the base of method of Encheva and Kiryakov /2002/. Part of obtained results is presented in table 5.

Table 5. Characterization of sunflower lines, obtained by interspecific and intergeneric hybridization for resistance to Phomopsis, Phoma, Alternaria and Sclerotinia, harvest 2009.

Accession, Pedigree	Resistance to,			
	Phomopsis	Phoma	Alternaria	Sclerotinia
	grades 0-4			grades 0-5
Sc-1 - 7115R /c.s. x C. acanth./	3	0	0	1
Sc-2 - L-6116B	1	0	0	2
Sc-3 - 7015R /c.s. x *H. debilis*/	0	0	1	2
Sc-5 - 7043R /c.s. x *H.pauciflorus*/	2	0	3	0
Sc-8 /c.s. x *H. argophyllus*/	0	0	0	0
Sc-9 /c.s. x *H. argophyllus*/	0	0	0	1
Sc-16 /c.s. x *Silfium* sp./	3	0	0	1
Sc-18 /c.s. x *Grindelia* sp./	3	0	2	1
Sc-23 /c.s. x *Telekia* sp./	2	0	1	0
Sc-27 /c.s. x *Inula* sp./	1	0	0	0
Sc-31 /c.s. x *Gaillardia* sp./	3	0	3	1
Sc-33 /c.s. x *Carduus* sp./	0	0	2	1
Sc-39 /c.s. x *Inula* sp./ x Tith.	2	0	0	1

Sc-51 /c.s. x *Carduus* sp./	1	0	2	1
Sc-53 /c.s. x *Tith.* sp./ x *Arct.*	1	0	3	1
Sc-56 /c.s. x *Arctium* sp./	2	1	0	1
Sc-60 /c.s. x *Tith.* sp./ x *Verbes.*	2	0	2	1
Sc-62 /c.s. x *Grindelia* sp./	1	0	2	1
Sc-62 /c.s. x *Zinnia* sp./	2	0	0	1

Creating new sunflower forms resistant to powdery mildew (*Erysiphe cichoracearum* D. C.).

Powdery mildew (*Erysiphe cichoracearum* D. C.) occurred first on the sunflower leaves surface. It covers them partly and then fully with fine white powdery bloom on the upper side. In suitable for pathogen conditions (in nurseries) plat stems covered too. Sometimes could be observed white bloom of infection in form of lump. Usually it could be observed in later planted sunflower plants.

For the conditions of our institute first symptoms of the infection during the period 1983-1987 was observed at about middle of August. In 1988 and 1989 the attacks were observed in June. Next years the infection was changed in its virulence and began at different period of time during the period June-August. In the conditions of the quarantine plots in 1988 from 101 accessions of annual species lack of infection was observed in only 7 accessions – two from *H. praecox* and *H. debilis* and three from *H. argophyllus*. From 102 perennial accessions lack of infection was observed in 14 of them. In most cases infection spread on all plants from an accession and reached grade 4. Full and high resistance showed accessions M 043 and M 006 from species *H. decapetalus*, *H. glaucophyllus* accession M 012, *H. resinosus* M 046, *H. tuberosus* M 004, *H. mollis* M 020 and M 034, *H. giganteus* M 011 and *H. debilis* subspecies *debilis* E 011.

As a result of crosses and obtained hybrid forms on the base of these accessions was established that the transferred resistance was controlled by a group of genes and for the two accessions of *Helianthus decapetalus* - by one dominant gene.

Now there are 30 accessions of hybrid forms maintained, resistant to the pathogen. Some of them - B and R lines, resistant to the broomrape parasite and to the downy mildew (table 1).

Creating new sunflower forms, resistant to the parasite broomrape (*Orobanche cumana* Wallr.).

Broomrape (*Orobanche cumana* Wallr.) causes annually great damages of the agriculture in the countries from the Mediterranean region, South-east Europe, Middle East and other regions (Cubero, 1986; Melero-Vera et al., 1989 and etc.). In Bulgaria it spread widely at 40s.

Broomrape is highly specialized obligate parasite with no capability for independent photosynthesis. It establishes a direct connection with the host and grows together with it. It forms a single unbranched stem. When it grows well its base is in a form of bulb. At the upper third of stem an inflorescence is formed with a great number of florets. The parasite is with great productive potential. One of its hosts is sunflower root. It appears on soil surface 50 days after germination of sunflower at a phase of complete button formation. It causes great damages of sunflower yield and seed oil content. Bachvarova (2004) pointed that broomrape infections causes in decrease of plants height with 6.4 %, head diameter with 27.8 %, andseed yield in high level of virulence decreases to 7 times. Comparatively high level of variability was eatablished - presence of 7 races: A, B, C, D, E, F and G. Shortly the new and more virulent races appeared (Knjazkov, 1950; Bachvarova, 1978a, b; Shindrova, 1994, 2006a and etc.). Several ways for preserving pathogen attacks were tested (some agro technical methods, herbicides, stimulators for stimulation of germ ability of seeds, biological agents and etc.), but no sufficient results were obtained till now. Up to now the only way for overcoming the parasite attacks is carried out by genetic ways.

In the official report of Dobroudja Agricultural Institute General Toshevo for 2008 is pointed that there are E, F and G races of broomrape on the territory of Bulgaria. In the south part of the country the new race G was observed, and in the north - races E, F and G. Appearance of the last two races in a short time aggravated the breeding work on sunflower.

During the last 20 years a great number of lines resistant to race E was developed. From 2004 the purposeful breeding was carried out for creating new lines resistant to race F, and from 2008 - to race G.

Hybrid materials resistant to race F and G were established and they were obtained from wild species *Helianthus decapetalus*, *H. tuberosus*, *H. pauciflorus*, *H. eggertii*, *H. divaricatus*, *H. maximiliani*, *H. praecox*, *Tithonia rotundifolia*, *Carduus acanthoides* and some others.

In tables 1, 2, 3, 6 and 7 results of investigations on the resistance to broomrape are presented. Many of the complete lines were included in hybrid combinations for developing of new hybrid cultivars. Lines 7009, 7015R, 7043R, 7115R and 6127B are parental forms of hybrid cultivars Maritza, Magura, Madan, Mesta and Musala.

Table 6. Characterization of sunflower lines, obtained by interspecific hybridization and resistant to broomrape, harvest 2006.

Accession, Pedigree	Resistance to,			Seed oil content, %
	Broomrape	Downy mildew	Phomopsis	
	%		grades 0-4	
7009R /c.s. x *H.tuberosus* 037/	100	100	2	47.21
7019R /c.s. x *H.praecox* 028/	100	100	1	47.85
7035R /c.s. x *T. rotundifolia*/	100	100	1	44.96
7041R /c.s. x *H.eggertii* 001/	100	100	1	50.15
7043R /c.s. x *H.pauiiflorus* 028/	100	100	2	51.14
7044R /c.s. x *H.pauiiflorus* 002/	100	100	2	49.97
7203R /c.s. x *H.decapetalus* 043/	100	100	1	48.07
7997R /c.s. x *H.delilis* 011/	100	100	2	46.25
C-23 /c.s. x *H.debilis* 011/	100	100	2	48.00
C-27 /c.s. x *H. H.tuberosus* 037/	100	100	2	52.16
C-28 /c.s. x *H. divaricatus* 044/	100	100	1	51.29
C-37 /c.s. x *H.debilis* 104/	100	100	1	47.11
6134B /c.s. x *H.debilis* 011/	100	100	1	47.95
6142B /c.s. x *H annuus* 004/	100	100	2	49.80
6874B /c.s. x *H.decapetalus* 043/	100	100	1	49.00
B1186n /c.s. x *H.eggertii* 001/	100	100	1	48.31
B1202n /c.s. x *H.decapetalus* 043/	100	100	2	52.27
B1206n /c.s. x *H.salicifolius* 045/	100	-	2	46.20
B1228n /c.s. x H.*argophyllus* 007/	100	100	1	47.55

Table 7. Characterization of sunflower lines, obtained by interspecific and intergeneric hybridization and resistant to broomrape, harvest 2009.

Accession, Pedigree	Generation	Resistance to, %		Seed oil content, %
		Broomrape	Downy mildew	
PR-1/8 /c.s. x *H.pauciflorus*/	23	100	100	48.48
PR-9/8 /c.s. x H.*tuberosus*/	25	100	100	47.27
PR-19/8 /c.s. x *H.divaricatus*/	19	100	100	45.25
PR-25/8 /c.s. x *H.pauciflorus*/	25	100	100	46.89
PR-35/8 /c.s. x *H.hirsutus*/	16	100	100	48.80
PR-41/8 /c.s. x *H.divaricatus*/	18	100	100	47.03
PR-47/8 /c.s. x *H.bolanderi*/	19	100	100	50.44
PR-56/8 /c.s. x *Aster speciosa*/	17*	100	100	49.56
PR-57/8 /c.s. x *Inula*/ x Tith.	16	100	100	50.35
PR-61/8 /c.s. x *Aster speciosa*/	17*	100	100	51.41
PR-63/8 /c.s. x *H.pauciflorus*/	25	100	100	48.80
PR-68/8 /c.s. x *Tithonia*/ x Verb.	16	100	100	48.36

Revealing of sources resistant to herbicides and developing of new B and R sunflower lines.

Sunflower growing is troubled because of great number of weeds as *Cirsium arvense*, *Convolvulus arvensis*, *Xanthium strumarium*, *Abutilon theophrasti* and etc. obstructive to normal sunflower growth. The struggle against them is difficult to be carried by the means of traditional technology and herbicides.

In searching the new means for destruction of these weeds the breeding is included. At present new cultivars and hybrids are used, resistant to the herbicides Granstar and Granstar express San (50 and 75 % active ingredient tribenuron), Pulsar 40 + Stomp 330 EK (40 g/l imazamox + 330 g/l pendimetalin), Pulsar 40 + arsenal 250 SL or intervix (40 g/l imazamox + 250 g/l imazapir) and etc. Using of these herbicides for hybrids such as PR64E83, Rimisol, Neomi, Armada and others leads to destroying of

almost all weeds.Using of these hybrids does not lead to appearance of phytotoxic characters and aloud recultivation of sowing area till full purity and to the harvest.

Searching of sunflower forms resistant to different herbicides began in 1996 in Bulgaria. In 2001 to the group of studied material some new ones were added. These are hybrid forms obtained from crosses between cultivated sunflower and several accessions of the wild species *Helianthus annuus* with origin North Dakota, Minnesota, USA.

Breeding work on creation of resistant to imidasolin and sulfonylurea hybrids began in 2005. The source for resistance used for this investigation was of J. Miller and K. Al-Khatib (2002). Many hybrid forms, obtained by interspecific and intergeneric hybridization were studied too. Our first sources for resistance to imidazoline were found in 2006. High percentage of resistant plants was obtained from the cross A123 x № ND 29. Resistant plants (about 20 %) were obtained from hybrids of № ND 18 and Arg 132. Sources for resistance were two accessions of wild *Helianthus annuus* - № ND 18, № ND 29 and one of *H. argophyllus* - № 132. Self-pollination and yearly treating of the selected material were carried out. In treatment with Pulsar 40 + Stomp 330 EK in 2009 of 21 plants from hybrid material of the cross L. 1607 x *Mdtricaria* sp., 17 plants were struck and 4 were slightly affected. For three of them a normal seed set was obtained. Studies in this direction still continued.

CONCLUSION

Great number of hybrid material was created, which was obtained by interspecific and intergeneric hybridization. Sources of resistance to six diseases and the parasite broomrape were found. New forms and lines, resistant to separate disease and the broomrape parasite or to two or more diseases and sometimes to broomrape were obtained.

Many of the lines were with high seed oil content and good combining ability. Five lines were parental forms of already registered hybrids. Three or four new sources for resistance to herbicides were established, and on that base the new hybrids were created.

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Results of sunflower breeding on resistance to broomrape on Don

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ABSTRACT

Results of many-years researches (1925-2009) for sunflower breeding on resistance to broomrape in Don Region are presented. The most virulent population of Orobanche Cumana Wallr. in north-western part of the Rostov region has been determined. Recommendations for reducing sunflower yield caused by broomrape are given.

Key words: sunflower – broomrape – breeding – varieties – hybrids – self-pollinated lines – virulence – commercial fields.

INTRODUCTION

A broomrape – Orobanche cumana Wallr. – is a plant parasitizing on sunflower root system. A broomrape exhausts all nutrients and water from the roots of other plants, and as result the plants become weaker, lose turgor, that leads to decrease in crop capacity, worsening of commercial seeds quality and in the most severe cases even to crop failure.

Different scientists such as botanists, physiologists, breeders pay attention to the problem of the broomrape harmfulness (Caspary, 1854; Koch, 1887; Byalosuknya V.V., 1919 (cited according to Byalosuknya V.V., 1930); Rihter A.A., 1928; Nechiporovich A.A., 1929; Pushkareva K.V., 1930; Beylin I., 1930; Novopokrovsky I.V., 1930; Pustovoit S.V., 1926; Zhdanov L.A., 1927, 1930 and etc.).

Scientist stated that sunflower and broomrape are in the continuous process of evolution – "host and parasite". Due to that fact the sunflower breeding on the resistance to broomrape is carried on permanently.

MATERIALS AND METHODS

If to address to the history of the sunflower breeding in Russia, in the beginning of the systematic scientific breeding the sunflower varieties, which were resistant to the existing in those years broomrape populations, were developed with the help of the method of individual selection (years 19910-1912). For example, Saratovsky 169, Saratovsky 206, (Plachek E.M., Steboot A.I., 1915), Kruglik A-41 (Pustovoyt V.S., 1926).

These varieties were cultivated widely. They took the leading position in areas of sunflower cultivation. The problem of broomrape seemed to be solved.

As S.I. Zhegalov noted (1924), the creation and introduction into production of broomrape-resistant sunflower varieties gave an opportunity to remove from the agenda the further work on struggle against this parasitic plant. But in the beginning and in the middle of the 1930s in a number of southern USSR areas there was a difficult situation with sunflower cultivation as this crop was massively infected by broomrape. The major oil-bearing plant was under serious threat and could have disappeared as an agricultural crop. In 1925 in these conditions and at such position of the crop L.A. Zhdanov started selection of sunflower on Don experimental station, established in 1924.

The inspections of industrial crops, held under supervision of L.A. Zhdanov in a number of areas showed, that all earlier developed varieties and landraces, as well as the samples from the world collection were affected by broomrape, and in some cases it led to the full crop failure. As noted V.S. Pustovoit (1970), L.A. Zhdanov independently from the works of experimental station "Kruglik" established the reason why the sunflower varieties were affected by broomrape.

As a result of these researches he found that different physiological races of broomrape parasitized on sunflower roots. They were named A and B. Moreover, broomrape B was more severe and it infected all the earlier developed varieties for 100%. For the first time the significant parental germplasm material on sunflower was collected in the Rostov region.

During the inspection of sunflower crops in Andreievsky and Oktyabrisky districts of the area of Mariupol (Donetsk region of Ukraine) L.A. Zhdanov managed to find individual plants which were broomrape-resistant. Applying a method of breeding on plot heavily artificially inoculated with broomrape seeds, for the first time L.A. Zhdanov determined broomrape-resistant sunflower plants which became a parental material for such varieties as Zhdanovsky 6432, Zhdanovsky 8281 and Stepnyak. In pre-war years these varieties were sowed on the area more than 1 million hectares. Production launch of these and other varieties allowed to increase considerably the productivity of sunflower, especially in broomrape infected areas, to expand areas under crops and to restore it as an agricultural crop.

It was proved, that the sunflower can give high yield of seeds and provide the oil production industry with raw material.

Such favorable conditions for sunflower cultivation existed for the period of 35-40 years. Crop rotation, usage of broomrape-resistant crops for sowing and performance of the demanded scientifically-proved technologies of cultivation allowed farmers to receive normal yields in all areas of sunflower cultivation.

In 1970-1974 years new signals from broomrape infected areas appeared. The inspection of field in the region carried by us and the estimation of varieties sown in areas infected by broomrape proved the appearance of new severer broomrape races (Gorbachenko F.I., Mezinova V.V., 1985; Gorbachenko F.I., Shurupov V.G., 1991). Disorder in crop rotation in sowing influenced greatly the process of new broomrape races appearance and their rapid spreading. Last years in separate farms the sunflower occupies up to 20% in crop rotation, and it leads to fast broomrape spreading and infection of the vast production areas.

Threat of decrease in sunflower crops because of affection by more virulent broomrape races caused the necessity of creation of resistant genotypes to this parasitic plant. In 1974 methodical researches were started at the station and the special field nursery where more than 30 kg of broomrape seeds collected in different areas of sunflower cultivation were sowed on 1 hectare. Annually this field nursery was infected with seeds of more virulent broomrape races, collected in different regions of Russia and Ukraine. As varieties-indicators were used the variety Kruglik A-41, which was resistant to the race A and non-resistant to the race B, and varieties Zenit and Mayak, which were resistant to the races A and B. During the estimation of the field nursery it was established that all examined sunflower varieties are infected by the new broomrape races in various degrees (tab. 1).

Table 1. Sunflower infected by new broomrape races (infected background)
Donskaya experimental station, 1976

Variety, No.	Quantity of broomrape flower stalks on 100 plants, pieces	Broomrape affection	
		%	degree
Zenit	2984	80	37.3
385	3192	80	39.9
Kirovogradsky 23	3311	70	47.3
Donskoy nizkorosly 47	5180	100	51.8
Zelenka 368	4949	90	56.1
Armavirsky 3497	5400	90	60.0
Mayak	6080	100	60.0
1141	4912	80	61.4
Peredovik	6300	100	63.0
319	4410	70	63.0
6843	5224	80	65.3
VNIIMK 8883	7230	100	72.3
Kruglik A-41 (control)	9157	100	91.6

Considering the dominant character of inheritance of broomrape-resistance, the sunflower plants, affected in a less degree, were put into paper isolator bags for self-pollination, intracultivar (intra-line) and intervarietal (inter-line) pollination. During researches it was established, that the most effective and productive method of reception of broomrape forms was self-pollination of sunflower plants on the

infected plot. As inbreeding leads to genetic differentiation of the parental material, after three years of work we managed to receive inbred lines, on which broomrape practically had no harmful effect.

The further selection work was also carried on the infected background. We picked out the plants with high resistance to this parasitic plant from the segregated populations. So, in 1976 from the early-ripe sunflower varieties were picked out 210 elite plants. The subsequent estimation and selection of generations of plants grown on the infected plot in the field conditions as well as their repeated estimation during the autumn-winter period in a greenhouse in accordance to A.J. Panchenko method (1975) allowed to increase resistance of these selections to more aggressive broomrape races from 4.2% up to 88.6% by 1982.

Simultaneously on the testing plots economic traits of progeny of the picked out elite plants were being studied. Their reproduction was being held on the space isolated plots of the directed pollination. The carried out methodical and practical researches allowed us to receive the new promising initial material with high (80-100%) resistance to a complex of broomrape races and possessing economic valuable traits (tab. 2).

During the next years the plot infected with broomrape was used for estimation, hybridization and breeding of a new parental material resistant to new virulent broomrape races. On creation of highly productive sunflower varieties resistant to new broomrape races, all parental material during all selection process (nurseries of the 1-st and 2-nd year of studying, preliminary and competitive test, nurseries of the directed pollination) passed an assessment on resistance to broomrape.

Table 2. Description of the best broomrape-resistant sunflower selections. Donskaya experimental station, (1981-1983)

Selections	Vegetation period, days	Seeds yield, t/ha	Seeds oil content, %	Oil yield, t/ha	Broomrape affection, % (infected plot)
Early-ripe group					
5714	93	2.94	48.4	1.33	4.2
3/174	96	2.91	51.5	1.42	7.4
5841	96	2.83	48.0	1.27	1.3
9061	93	2.73	50.5	1.30	0.0
Zenit (standard)	97	2.66	52.7	1.32	94.8
NSR_{095}		0.18			
Mid-season group					
10565	100	3.02	52.1	1.50	3.3
10470	101	2.90	51.1	1.41	0.0
10537	99	2.85	50.8	1.37	0.0
Mayak (standard)	103	2.72	52.2	1.35	99.7
NSR_{095}		0.21			

In the course of sunflower breeding for heterosis, creation of the self-pollinated lines was carried out on the field without broomrape during the 1st year, and then, since J_2, on the infected plot. On this area 2500-2700 lines of sunflower which passed the estimation on resistance to broomrape were sowed annually, and the best varieties according to broomrape-resistance and other traits were exposed to self-pollination or intralinear pollination. Using broomrape-infected plot and applying methods of self-pollination and intralinear pollination, the self-pollinated lines of sunflower VD340, VD342, VD1137, VD53, VD110, VD461, VD62, VD151 which combined high resistance to broomrape with other valuable traits from the selection point were received.

Application of the continuous control of a parental material on broomrape resistance, by its estimation on the infected plot in field conditions and in greenhouse conditions with its simultaneous studying in nurseries and reproduction in plots of the directed pollination, allowed us to create a parental material valuable for breeding practice. On its basis such varieties as Don 60, Azovsky, Kazachy, Donskoy large-seeded were developed as well as hybrids Donskoy 187, Donskoy 342, Orion, Kaskad, Donskoy 1448, Donskoy 151, Fermer, Mechta, etc., which are resistant to broomrape.

Besides application of self-pollination and intervarietal crossings of the selected samples allowed us to receive complex populations, which separate biotypes had high mass of 1000 seeds and according to this indicator almost did not differ from Armenian forms of ecotype (K-1589), but from the view of other economic traits represented practical interest for selection. As a result of sibbing of such biotypes were

separate plants with mass of 1000 seeds 110-160g and with low oil content in a kernel. Seeds of the selected plants were sowed on the isolated plots and were ancestors for the first Russian large-seeded variety for the confectionery industry – the variety "Donskoy large-seeded".

According to the results of competition between different sunflower varieties, the variety "Donskoy large-seeded" was infected by broomrape race B only on 24.1% and poorly by downy mildew, verticilliose, sunflower moth (tab. 3). It also fitted for harvesting.

Table 3. Results of assessment of Donskoy large-fruited variety on resistance to broomrape and diseases Donskaya experimental station, (1987-1988)

Diseases	Background	Infection, %	
		Variety "Donskoy large-fruited"	Variety-indicator
Broomrape A	artificial	0	100
Broomrape B	-//-	24,1	100
Downy mildew	-//-	30,9	100
Gray rot	natural	0	
White rot	-//-	0	
Dry rot	-//-	0,3	
Verticilliose	-//-	0	

Variety-indicator on broomrape – line VD 105 Rf
Variety-indicator on LMR – Donskoy nizkorosliy 47

On the basis of the held methodical researches the scheme of the rapid creation of confectionery sunflower varieties which were resistant to a complex of broomrape races was worked out. In vegetative-climatic chambers of the VNIIMK phytotron elite plants of Donskoy large-seeded variety were assessed for broomrape resistance and broomrape-resistant plants were selected (V.I. Klyuka, 1990) during the autumn-winter period. Among the 1000 seeds of Donskoy large-fruited variety that were given to us in 1991 for the reproduction, 46 broomrape resistant plants were sorted out during the autumn-winter period and 7 broomrape resistant plants were sorted during the winter-spring period. Next-years progenies of the best elite plants were sowed on an infected plot and on the directed pollination plot. As a result of this work in short term it was possible to raise the resistance of Donskoy large-seeded variety to a complex of broomrape races up to 85-95 %.

During the years of Perestroyka the works on sunflower breeding on resistance to broomrape were weakened. Unjustified expansion of sown areas under sunflower, delivery of seeds of varieties and hybrids that were not checked on the resistance to local broomrape populations, disturbances in crop rotation, the excessive interest for surface tillage, etc. promoted occurrence and distribution of more virulent broomrape races and to decrease in a crop of this valuable oil-bearing crop. During the last years only in the Rostov region the area under sunflower crops is 1.1-1.3 million hectares, instead of 450-550 thousand hectares in accordance with the scientifically-proved systems of agriculture.

Absence of efficient measures of struggle with broomrape led to that during many years in the most favorable areas for sunflower cultivation huge stocks of broomrape seeds were collected in soil. Keeping the viability within 8-10 years they are capable to infect the sunflower plants.

The existing situation with sunflower made us to assign a field area in a crop rotation for creation a broomrape-infected field. There was a task for breeders, working on the station, to determine percentage and extent of broomrape infected sunflower varieties and hybrids, which were developed in our station and in the other breeder institutions of our country and abroad and which were sowed in the Rostov region and in the South Federal district of Russia.

For creation of the broomrape-infected field there were improved the methods and techniques of broomrape plants gathering, their transportation, thrashing, seeds cleaning and their storage, and their further use for sowing.

Broomrape seeds were collected from the sowing areas of foreign and domestic selected sunflower varieties and hybrids in Azovky, Kagalnitzky, Zernogradsky, Myasnikovsky and Neklinovsky districts of the Rostov region. The collected seeds were united in one sample under the name Priazovskaya. Broomrape seeds collected from the sunflower sowing areas in Morozovsky, Tatzinsky, Konstantinovsky districts were bulked under the name Morozovskaya. To provide even sowing of broomrape seeds on a field we prepared beforehand a working mix of broomrape seeds, sand, ground and fertilizers.

In autumn before sowing of broomrape seeds the field was divided into 4 parts:

1 – a plot without broomrape seeds
2 – a plot with broomrape seeds of Priazovskaya population
3 – a plot with broomrape seeds of Morozovskaya population
4 – a plot with broomrape seeds of Morozovskaya and Priazovskaya populations mixture.

In spring presowing cultivation of each part of the field according to the plot was done together with marking 70x70 cm and sowing of seeds of the studied varieties and hybrids. Handling of plants, manual weeding with simultaneous formation of density of plants standing (1 plant in a hill), as well as phenological supervision, accounts, measurements were being held during plants vegetation.

In total in year 2009 137 varieties, hybrids of domestic and foreign development, as well as of Donskaya station breeding, were estimated on the fields infected by populations of broomrape seeds. During the period of sunflower vegetation the "formation of a head-bloom-ripening" was counted on increasing susceptibility of plants varieties, hybrids and lines of sunflower to different broomrape populations.

It has to be noted, that an assessment of 31 varieties and hybrids of sunflower was held in 3 multiple frequencies on four backgrounds, infected by the different broomrape populations.

As T.S. Antonova stated (2009), the international classification of broomrape races which today includes races A, B, C, D, E, F, G and H was accepted. For their recognition it is necessary to have the tested varieties or lines-differentiators for each broomrape race. The station doesn't have such samples, therefore at the given stage of the researches we use the term "a broomrape population".

Results of an assessment of sunflower varieties and hybrids on various broomrape-infected plots showed different percentage and a degree of infection.

If to compare virulence of Priazovskya and Morozovskaya broomrape populations, almost on all studied varieties and hybrids showed the smaller percent of infection by Priazovskaya broomrape population. The least percentage of infection showed the following varieties: Donskoy 60 – 32 %, Saratovsky 85 – 34.1 %, Saratovsky 20 – 55 %, Maria – 15.8 %, Spartak – 30.7 %, Albatros – 33.3 %, Hermes – 48.8 %, Kozachy – 26.2 %, Buzuluk – 21.9 %, Lakomka – 45.4 %.

On the average examined varieties and hybrids were infected by Priazovskaya broomrape population for 50,4 %, and by Morozovskaya broomrape population for 73,3 % at a degree of infection 6,5.

This indicates that Priazovskaya broomrape population has less virulent races in its structure.

Studying of a line material for the resistance to this parasitic plant showed, that all lines that had been breeded on the station earlier, were infected by broomrape in a different degrees. The least quantity of flower stalks of broomrape was found on lines: VD 1448 – 7.9 % of infection, VD 344 – 11.6 %, though they were considered earlier to be genetically resistant to broomrape. Line ED 95 – 16.0 % of infection, ED 269 – 26.6 %. Other lines were infected much more severe: VD1137 – 74.5 %, ED 803 – 77.4 %, VD 151 – 51.9 %. The test and assessment of a new line material allowed to reveal and select separate line plants which poor infected by more virulent Morozovskaya broomrape population (tab. 4).

Table 4. Self-pollinated lines, which are best for broomrape resistance
Donskaya experimental station, 2009

Line index	Broomrape infection	
	%	degree
J-3/227	0	0
J-3/224	0	0
J-3/261	0	0
J-3/299	25.0	1.0
J-7/671	0	0
J-5/1276	0	0
J-8/1933	12.5	2.0
J-8/1910	0	0
J-8/1977	16.6	4.0
VD 22 – control	100	5.0

From these lines there are 4 parent (J-3/227, J-3/224, J-3/261, J-3/299) and 5 Rf-lines (J-7/671, J-5/1276, J-8/1933, J-8/1910, J-8/1977). In 2008 in accordance with A.D. Panchenko's method assessment in greenhouse conditions these lines showed resistance to broomrape. We will continue to work with them.

During next years we plan to continue works on creation of a parental broomrape-resistant material as well as on inspection of sunflower crops in all soil-climatic zones of the region in order to find more virulent races and populations of broomrape.

RESULTS AND DISCUSSION

For reduction of the harmful effect of this parasitic plant on commercial fields in the Rostov region in view of the existing situation the following measures on sunflower cultivation have been developed and offered to farmers:

1. to follow strictly alternation of crops in a crop rotation, returning sunflower crops on a former field in 8-10 years.
2. to limit by administrative measures sunflower cultivation on the area which is not exceeding 15 % from the whole arable area (the Regulation of Administration, the Rostov region, No. 182 dated 16.04.2009).
3. to reduce the surface soil treatment on the fields which will be used under sunflower crops as it leads to accumulation of the infectious beginning pathogens, pests, broomrape in the top layer of soil (0-15 cm) and to replace it with deep plowing with a turn of a layer.
4. fields where the return is forcedly conducted on the 5th - 6th year shall be sown landraces seeds as they are more adapted to local conditions resistant to broomrape.
5. the hybrid seeds of foreign selection should be tested for broomrape resistance on infected plot and for illnesses in the research institutions and on the state variety test plots before import to Russia.
6. to carry out widely industrial tests of foreign sunflower hybrids for resistance to herbicides imidazoline groups and to Euro-Light herbicide and to ascertain the financial viability of their cultivation.
7. to use binary sowing of sunflower together with legume crops, such as melilot and tare as there crops allow not only to struggle with broomrape, but also to increase sunflower productivity.
8. for reducing the density of infection of virulent broomrape races on the fields one should sow seeds of varieties and hybrids, which are not resistant to this parasitic plant. To the time of flowering, sunflower plants should be mown and could be used as green manure.

Carried out researches proved again, that with the most reliable and effective way and method of gaining the parental broomrape-resistant material is breeding on the basis of which varieties and hybrids resistant to more virulent broomrape races and populations will be created,.

Their introduction into the crop production as well as performance of elementary technologies of cultivation will allow keeping sunflower as highly productive and profitable oil-bearing culture.

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Development of sunflower genotypes resistant to downy mildew

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ABSTRACT

Sunflower is attacked by more than 40 different diseases of which only a certain number causes serious reduction of seed yield. One of the most damaging diseases is downy mildew which is caused by *Plasmopara halstedii* fungus (Farl.) Berl.et de Toni (syn. *Plasmopara helianthi* Novit.). Sunflower downy mildew has a great economic importance in all countries where sunflower is grown. When the meteorological conditions during the vegetation period of sunflower become favourable for disease development, the damages produce considerable reducing of the seed yield and the oil content.

The best way of controlling the fungus is to grow resistant cultivates and because that the major objective of this study was to developed sunflower genotypes genetically resistant to dominant races of downy mildew in Serbia. During this work two co-dominant CAPS markers for *Pl-6* gene were developed which can also be used for *Pl-7* gene. For introduction of these genes in breeding program marker assisted selection (MAS) was used. Developed commercial sunflower inbred lines exhibit resistance to all known races of downy mildew in Serbia indicated incorporation of resistance to downy mildew in well-known and widely produced hybrids. Except that, *Pl-genes* were introduced to large number of new inbred lines and new downy mildew resistant hybrids. These new hybrids reach higher seed and oil yields then hybrids widely produced.

Key words: downy mildew – hybrid – inbred line – race – sunflower

INTRODUCTION

Diseases are the main limiting factor in the production of sunflower (*Helianthus annuus* L.) and they cause poor realization of genetic yield potential of sunflower hybrids. Downy mildew is an economically significant disease. It is caused by the fungus *Plasmopara halstedii* (Farl.) Berl. Et Toni. (syn. *Plasmopara helianthi* Novot.). Downy mildew is widespread in all sunflower-growing countries with the exception of Australia. According to Tikhonov (1975), it had been first discovered on sunflowers in the United States in 1883, and in 1892 it was found on *Helianthus tuberosus* in Russia. As the sunflower expanded to other countries, the disease followed it closely, especially after the World War II. The rapid expansion of the disease may be explained by its transfer with infected sunflower seed. In the former Yugoslavia, it was discovered Perišić (1949), and described by Nikolić (1952).

Downy mildew has large economic importance in all sunflower-growing countries. Its occurrence depends on the growing and climatic conditions during sunflower growing season. If the season is rainy, the number of diseased plants increases in proportion with the number of rainy days. The number of infected sunflower plants may vary from 1 to 100%. Extent of damage depends on infection type, i.e., whether it is primary (systemic) or secondary infection. While the primary or systemic infection causes significant yield reductions, secondary infection has no importance for the production of sunflower (Aćimović, 1998).

Primary infection is effected during seed germination in the soil and the emergence of sunflower seedlings. It may be caused by fungus mycelium or oospores present on infected seeds, or by oospores present in infected soil into which healthy seeds were sown. The number of diseased plants depends on the amount of inoculums on seeds and in soil. No matter if primary infection starts from seeds or soil, the course of disease development in infected plants is identical. The fungus develops in unison with the development of young plants. It penetrates the root, stem, cotyledons and reaches the meristematic tissue at the top of young plants. The fungus develops inside the infected plants intercellularly, in all plant parts, pervading the young tissues and depriving the infected plants of assimilates and water. This is why infected plants lag behind healthy ones in growth and development. This way of fungus expansion inside the plant tissues is called a systemic infection. It begins with the infection of the germ and ends with the infection of the head and seeds. The fungus penetrates all parts of the seed (husk, endosperm and germ) which then produces a new infected seedling. In that way, conditions are created for the occurrence of the disease in the subsequent sunflower growing season. Infected plants, in addition to having stunted growth, i.e., short internodes, are chlorotic and with a platform head which gives a smaller yield than the normal

head. The infected plant parts, the root, cotyledons, the stem, and especially the leaves, there occurs abundant white mycelium, which is typical for this disease. The mycelium occurs also on the reverse side of the leaves and it contains the vegetative organs of the fungus - conidiophores and conidia (zoosporangia). On the upper side of the leaf persons there occur chlorotic spots. Infected plants collapse and remain in the field after harvest. Numerous oospores that form on these prostrate plants are overwintering organs of the fungus, which are ready to start a new cycle of infection in the spring.

In the course of sunflower growing season, spots occur on the aboveground plant parts, especially on leaves. The spots are polygonal, with a characteristic white mycelium on the reverse side of the leaf and chlorosis on the upper side. These spots originate from summer conidia (zoosporangia) and they represent a secondary infection. The intensity of their occurrence is typically low and they have no important impact on sunflower yield.

Measures of protection against downy mildew include cultivation practices, chemical measures and the use of resistant hybrids. The recommended cultivation practices are the use of healthy seeds for planting, seed treatment with fungicides against downy mildew, proper crop rotation, i.e., intervals of 4-5 years between two sunflower crops in the same field, selection of fields for sunflower growing that are at least 500m away from a field planted to sunflower the previous year, because of infected harvest remains in that field, removal of volunteer plants, sowing at optimum time and avoiding late planting, and deep plowing of the field after sunflower harvest.

The most effective chemical measure of downy mildew control is seed treatment with metalaxyl-based preparations. This measure protects the sunflower crop at the time of the primary infection, i.e., at early stages of development of sunflower. In addition, various chemicals for post-emergence treatment are available, but this practice raises the question of economic feasibility.

Use of genetically resistant hybrids is definitely the most effective way of controlling downy mildew in sunflower. Therefore, experiments have been set up with the objective of developing sunflower genotypes genetically resistant to dominant race of downy mildew in Serbia.

MATERIALS AND METHODS

In these experiments we used a part of sunflower breeding material developed at Institute of Field and Vegetable Crops in Novi Sad in the period from 2001 to 2009.

The following inbred lines developed in USDA-ARS Sunflower Research Program, Fargo, North Dakota, USA, were used as donors of downy mildew resistance genes:

1. B-lines: Ha-336 (*Pl-6*), Ha-338 (*Pl-7*)
2. Rf-lines: RHA-340 (*Pl-8*), RHA-419 (*Pl-arg*)

Ha-26-PR (*Pl-6*) and JM-8 (*PL-6* +), B-lines of sunflower developed in an earlier program at Institute of Field and Vegetable Crops were used as additional donors of downy mildew resistance genes.

The following donor lines developed at Institute of Field and Vegetable Crops had good GCA and SCA and high tolerance to *Phomopsis*:

1. B-lines: CMS-1-90, Ha-48, VL-A-8, CMS-1-50, PH-BC1-92, PH-BC1-74, Ha-981
2. Rf-lines: RHA-583, RHA-SES, RHA-N-49, RHA-168, RHA-SNRF, RHA-RU-3, RHA-576

In the first year of the experiment, above mentioned sunflower inbred lines were crossed with each other, each B-line resistant to downy mildew with each B-line tolerant to *Phomopsis* and each Rf-line resistant to downy mildew with each RF-line tolerant to *Phomopsis*. The plants that served as the female component in the crosses were manually emasculated in the early morning hours, before opening of anthers. After these initial crosses, the program of development of sunflower genotypes resistant to downy mildew was divided in two parts. The first part was aimed at the development of completely new inbred lines resistant to downy mildew using the head-to-row pedigree method of selection. The second part involved the conversion of commercial inbred lines into a form resistant to downy mildew using the backcross method. Winter greenhouse was used in both parts of the programs to speed up the selection process and obtained three sunflower growing seasons in a calendar year.

After harvest of each generation, individual plants were analyzed for resistance to downy mildew. The generations grown in field were also assessed for resistance to *Phomopsis*. Resistance to downy mildew was tested by a laboratory method (Lačok, 2008), using the mildew race 730. In the course of this work, two co-dominant CAPS markers for *Pl-6* were developed (Panković et al., 2007) which were later found to be applicable for *PL-7* too (Saftić-Panković et al., 2007). Marker assisted selection (MAS) was used for introgression of these genes, which was of great importance especially in the program of backcrossing.

RESULTS AND DISCUSSION

For a long period of time there existed only two races of downy mildew. Race 100 was present exclusively in Europe and race 300 was present in North America. Fick and Zimmer (1974) found that gene *Pl-1* controlled the race 100 while gene *Pl-2* controlled both races, 100 and 300. These two genes controlled the downy population in Europe until 1998 when new races emerged (710 and 703) in France (Tourvieille de Labrouhe et al., 1991). Later research showed that these races were introduced from the USA via infected seeds (Roeckel -Drevet et al., 2003). After the introduction of these races, there occurred a conflagration of new downy mildew races, especially in France. Those were race 304 (Tourvieille de Labrouhe et al., 2000), and races 300, 307, 314, 700, 704 and 714 (Penaud et al., 2003). According to Viranyi (2008) to date has revealed 35 different races of downy mildew have been discovered so far, of which only five are prevalent (300, 330, 710, 730 and 770). A special problem is the occurrence of new downy mildew races in France and Spain that are resistant to metalaxyl, the main active substance that controls downy mildew (Albourie et al., 1998, Molinero Ruiz et al., 2000).

In Serbia, only race 100 was present until 1990. However, the isolate sampled in 1991 under the name of NS 912 was identified as race 730 and at that moment it comprised about 10% of all isolates. Already in 1996, this race made about 50% of the total isolates (Maširević, 1998). Today, race 730 is definitely dominant in Serbia (Lačok, 2008).

Genes of resistance to new races of downy mildew have been determined in wild sunflowers and they have been transferred into cultivated sunflower genotypes. Resistance to downy mildew is controlled by several single dominant genes called *Pl-genes*, which are racially specific and which provide vertical resistance. More than ten of such genes have been discovered so far. The safest method of combating the fungus is entering resistance genes in sunflower hybrids.

As the *Pl-genes* are race-specific, there is a great probability that their resistance will be overcome by new races of downy mildew in a relatively short period of time. To prolong resistance duration, attempts have been made in breeding programs to combine as many different resistance genes as possible. Genetic studies of *Pl-genes* indicated that they are present in at least three gene clusters (Vear, 2004). Genes *Pl-1*, *Pl-2, Pl-6, PL-7* and *PL-7* + are in the first cluster, genes *Pl-5* and *Pl-8* in the second and genes *Pl-4* and *Pl-arg* in the third. The following B-lines were selected donor lines from cluster 1: Ha-336 (*Pl-6*), Ha-338 (*Pl-7*) and JM-8 (*Pl-6* +). Rf-line RHA-340 (*Pl-8*) was selected from the cluster 2, and RF-line RHA-419 (*Pl-arg*) from cluster 3. The last two lines are the donors of genes *Pl-8* and *Pl-arg* which for the moment provide resistance to all known downy mildew races. The above inbred lines were selected for donors in consideration of their genetic origin. Namely, the line Ha-336, the donor of gene *Pl-6*, had been transferred from wild *Helianthus annuus*, the line of Ha-338, the donor of gene *Pl-7*, had been transferred from *Helianthus praecox*, and the lines RHA-340 and RHA-419 are donors of genes *Pl-8* and *Pl-arg* which had been transferred from different populations of *Helianthus agrophyllus*. By introducing genes *Pl-6*, *Pl-7* and *Pl-6*+ into the female lines and genes *Pl-8* and *Pl-arg* into the male lines, we shall be able to develop sunflower hybrids resistant to all races of downy mildew in the long run.

The inbred lines used in this research as donors of resistance to downy mildew, i.e., the donors of genes *Pl-6*, *Pl-7*, *Pl-8* and *Pl-arg*, are characterized by poor agronomic characteristics. In the first place, they are highly sensitive to *Phomopsis*. Therefore, these lines had to be crossed to inbred lines made at Institute of Field and Vegetable Crops, Novi Sad, which are characterized by good agronomic characteristics and high tolerance to *Phomopsis*. A portion of the obtained F_1 generation was selfed in order to obtain the F_2 generation, and the other portion was crossed with inbred lines known as donors of resistance to *Phomopsis*. The F_2 generation was then subjected to the pedigree method of selection in order to create new genetic variability and thus create the initial population for the selection of new inbred lines resistant to downy mildew. In the second part of the selection program, commercial B-lines were backcrossed in order to be converted them into a form resistant to downy mildew.

Using the discovered co-dominant CAPS markers for *Pl-6* gene allowed us to identify homozygous plants resistant to downy mildew in each generation of selfing. In addition to the analysis of gene presence, resistance was also checked by analyzing the response to primary infection with fungus spores. Selfing and the mentioned analysis were done to a complete stabilization of this trait, i.e., until the lines became homozygous for resistance to downy mildew. Additionally, each generation of selfing grown in field conditions was selected for resistance to *Phomopsis*. Oil content in seed was also analyzed. Based on the obtained results, we selected new inbred lines resistant to downy mildew and highly tolerant to *Phomopsis* (Table 1).

Table 1. Newly developed sunflower inbred lines possessing different *Pl-genes*

Source of genes	No. of lines	Type
Ha-336 (*Pl-6*)	267	B-line
JM-8 (*Pl-6+*)	37	B- line
Ha-338 (*Pl-7*)	62	B- line
RHA-340 (*Pl-8*)	206	Rf- line
RHA-419 (*Pl-arg*)	103	Rf- line

The multi-year work at the Novi Sad Institute on resistance to downy mildew has produced significant results. Resistance genes have been introgressed into a large number of lines in the program of backcrossing. As a result of these research efforts, in 2009, the hybrids VELJA, KAZANOVA and RIMI-PR were commercially grown in the form resistant to all races of downy mildew present in our Sebia.

Simultaneously we developed a new genetic variation, i.e., lines resistant to downy mildew. New female and male lines possessing different *Pl-genes* have also been developed. These lines had already been crossed and new sunflower hybrids resistant to all races of downy mildew present in Serbia were developed, SREMAC, DUŠKO and a high-oleic hybrid OLIVA. The hybrids SREMAC and DUŠKO deserve to be singled out for their high yields of seed which make them leading hybrids not only in Serbia but also in other European countries. The new lines with different *Pl-genes* make it possible to develop new sunflower hybrids, which may provide a long-term solution to the problem of downy mildew control.

CONCLUSION

A breeding program aimed at the development of hybrids resistant to downy mildew has produced genotypes with high values of agronomically important characteristics. The obtained results allowed us to draw the following conclusions.

- The discovered co-dominant CAPS markers for *Pl-6* gene of resistance to downy mildew can be used for efficient identification of plants resistant to downy mildew race 730 in selection material originating from various genetic sources.
- Commercial sunflower lines have been converted into forms resistant to all downy mildew races present in Serbia, which allowed us to convert extensively grown sunflower hybrids VELJA, KAZANOVA and RIMI-PR into forms resistant to downy mildew.
- *Pl-genes* have been incorporated in a number of new inbred lines and new hybrids have been developed which are resistant to downy mildew. These are: SREMAC, DUŠKO and OLIVA. The new hybrids have higher yields of seed and oil compared with the currently most widely grown hybrids in Sebia.

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Results of sunflower breeding for resistance to disease complex and broomrape

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ABSTRACT

The paper shows the results of many-year activities on the creation of a new generation of sunflower hybrids, high-yielding, resistant to main diseases' pathogens. The system of infection backgrounds developed in Plant Production Institute nd. a. V. Ya. Yuriev of Ukrainian Academy of Agrarian Sciences is an inseparable part of the process of sunflower hybrid breeding. The system includes field and laboratory investigations on resistance to the most harmful diseases of sunflower on natural, artificial and provocative infection. Immunological evaluation and differentiation of the breeding material as to the resistance to the pathogens of phomopsis, bud-rot and cottony rot and broomrape under artificial field infection, green-house-field method of infection and sunflower infection evaluation with false downy mildew's pathogen and broomrape have permitted to identify the variety-samples – sources of genetically conditioned resistance to main pathogens. The immune and weakly infected variety-samples are recommended as the donors of resistance to the pathogens by carrying out back crosses and following strict selection on infections backgrounds. Special attention is paid to the problem of combination of the resistance to disease pathogens and high indices of productivity in one genotype. The evaluation of the perspective material in different soil-climatic zones of Ukraine within the program's fulfillment on ecological variety testing has given an opportunity to choose the hybrids, which demonstrated stable resistance to biotic environmental factors. The sunflower hybrids recommended for cultivation in Ukraine, Russia and Belarus with group resistance to disease pathogens and broomrape have resulted from many-year activities.

Key words: breeding – broomrape – pathogen – resistance – sunflower

INTRODUCTION

Sunflower breeding for high potential productivity and resistance to biotic environmental factors ensures not only the increase of gross produce production, but also increases its quality considerably, ecological balance, secures the environment and purity of crop products. In Plant Production Institute nd. a. V. Ya. Yuriev of Ukrainian Academy of Agrarian Sciences the problem of hybrid development with an optimal level of genetical control of the production process and produce quality formation is being solved on the systemic approach basis, i.e. by combining the researches of breeders, geneticists and immunologists. Owing to specialists' cooperation in different science areas it has became possible not only to develop the system of infections backgrounds, but also to make it an integral part of the sunflower hybrid breeding stages. Many-year activities of the institute's scientists on the improvement of methods for creating infection backgrounds in field and laboratory conditions, the methods for estimation and selection of resistant forms, the provision of an optimal infection level on breeding material enabled them to introduce such a system. The development of the main well-grounded principles for sunflower heterotic breeding, the study on the inheritance of resistance to the pathogens of mostlyspread diseases and broomrape in the Eastern Forest-Steppe of Ukraine have served as theoretical grounds for creating original material and hybrids of sunflower, in which high indices of productivity and resistance to diseases' and broomrape's complex were combined [1].

MATERIALS AND METHODS

The system of infections backgrounds functioning at Plant Production Institute nd. a. V. Ya. Yuriev of UAAS (Fig. 1) has been developed for estimation and constant selection of the breeding material for resistance to diseases' races and isolates, which are the most harmful in the North-Eastern Forest-Steppe Zone of Ukraine, as well as not typical for the zone of the Kharkov Breeding Centre.

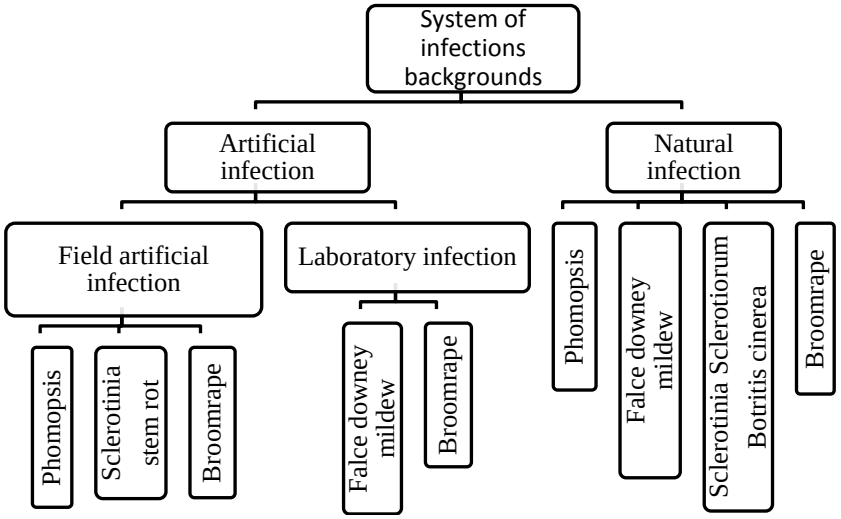

Рис. 1. System of infections backgrounds in sunflower, Plant Production Institute nd. a. V. Ya. Yuriev of UAAS

The evaluation of sunflower for resistance to false downy mildew's causal agent in the laboratory is conducted by the express-method [2], to broomrape – according to the method [3]. The infection field site for Sclerotinia stem rot agent is created in accord with the method [4]. The infection field site for phomopsis – according to the synthesized practices by Russian [5], Serbian [6] and French [7] researchers.

Weight estimate of symptoms of phomopsis and head rots manifestation is carried out on the basis of the method [8]. The weighted mean of disease severity and its spread is measured according to the method [9].

The estimation on tolerance of hybrids to soil infection caused by Sclerotinia stem rot is conducted by calculating the number of healthy sprouts in per cent as for the number of sown seeds, with correction of field germination, which is estimated on the control plots. The division of experimental materials into groups is conducted as to an infection level, with provision for a degree of confidence – $LSD_{0,05}$ [10].

The level of natural infection is expressed as mean value of affection of the aggregate of the aggregate of varietysamples. The parameter „mean value per experiment" is considered as basic for quantitative estimation of disease's phenotypic manifestation.

RESULTS AND DISCUSSION

The investigations on search and studies of sunflower biotypes resistant to Sclerotinia stem rot, grey stem blight, false downy mildew and broomrape at Plant Production Institute nd. a. V. Ya. Yuriev of UAAS have been being carried out since the 60-ies of the 20-th century. At that time complete absence of plant resistance to many pathogens of sunflower diseases made the researchers to look for the sources of resistance among wild relatives of *Helianthus*. A specific task was set before the scientists, that is, to create initial material by interspecific hybridation, which could combine complex immunity to pathogens of main fungal diseases and broomrape possessing the productivity at the level of bred cultivars of sunflower, which were spread during those years. The sources of group immunity to main pathogens of sunflower were identified by crossing sunflower with annual and perennial diploid (2n=34), tetraploid (2n=68) and hexaploid (2n=102) spp. (12 species in total) [11].

In the outcome of 10-year experience (1961-1971 ys) some interesting results were obtained and general theoretical conclusions were made as to a possible use of interspecific hybridization in breeding sunflower for group immunity [12]. On the basis of many-year investigations it was concluded that for the enrichment of sunflower genepool the hexaploid group of species being distinguished by group immunity to *Plasmopara helianthi* N., *Puccinia helianthi* Schw., *Phoma* sp., *Orobanche cumana* Wallr., *Sclerotinia sclerotiorum* (Lib) de Bari. [13] is of a great interest. With the method of interspecific hybridization, which had combined back- and pair crosses with multiple individual selection under severe infection, a great variety of the forms with a different degree of resistance to broomrape, grey rot and *Sclerotinia sclerotiorum* varying by economic traits were developed. On their basis in the 80-ies the breeding

material adapted to the local growing conditions, being characterized by high productivity and oil content by resistance to local pathogenic races, high combining ability was created in the institute. Now this material is being widely used in sunflower commercial hybrids.

Since 1981 the investigations on search and studies of sunflower biotypes resistant to grey and white rots' pathogens have got a new approach in the institute by using breeding material of a new type and the improved methods for resistance estimation [14, 15, 16]. The specimens resistant to white rot are identified both among collection and breeding materials. As regards to the results of samples' immunological tests in connection with grey rot it has been stated that resistance to this pathogen depends considerably on the weather and crop vegetation conditions. The data obtained from 1981 show presence of differentiation as to resistance to grey rot's pathogen in sunflower varietysamples, however, this resistance to a considerable extent depends on weather conditions at the crop vegetation period.

Since 1990 the search of resistant forms and the study of a new pathogen of phomopsis have been launched [17]. In field and green-house conditions the estimation of varieties, hybrids, experimental hybrid combinations and their parental lines was made. During 1992-1998 ys sunflower varieties and lines were estimated in order to reveal potential sources for productivity and resistance to disease pathogens. The lines recommended for usage in the breeding programs ensured high resistance of hybrids developed on their basis to phomopsis.

For the purpose of speeding up the creation of sunflower hybrids resistant to broomrape there were the problems connected with breeding material evaluation in the chambers of the green house which had to be studied by breeders and immunologists, that is: parasite severity, the number of plants studied per pot, the influence of storage terms of broomrape, as well as, of lighting period on affection under artificial infection [3, 18].

High variability of harmful pathogenic microorganisms makes breeders and immunologists to select continuously the sunflower forms being resistant to them to use in practical breeding. The last 10 year (2000-2010) results of the investigation are indicative of effective use of the system of infectious sites created and up-dated in the institute in sunflower line material and hybrid breeding.

At present for the selection of the forms resistant to broomrape under artificial climate and field artificial sites the more aggressive isolate of this plant-parasite from Donetsk Oblast is being used. The estimation of the breeding material with different degree of inbreeding on the natural infectious site is made yearly with sorting of all affected samples. In order to distinguish resistant forms among morphologically homogenious materials the green-house conditions are used, that is combined with resistant plants' selection on artificial infections site under field conditions. Long-term estimation of sunflower breeding material by the improved method permitted to create a working collection of resistant selfed lines of a maternal type, on the basis of which the future selection-genetical investigations are carried out. The collection includes the specimens, which are immune to broomrape, and with low degree of affection (0-10 %), as well, i.e. perspective for the next selection. The immune lines are included in the program of inbred crosses.

Phytopathological estimation of the collection with the lines-restorers of fertility as to resistance to false downy mildew's pathogen under natural conditions according to standard scoring scales has shown that 92,1-96,4 % of the total number of lines have a very high resistance – 9 scores provided with a continuous selection of the resistant samples (Table 1).

Table 1. Differentiation of the collection of lines-restorers of fertility as to the pathogen of false downy mildew in sunflower, natural site, 2008-2009 ys.

Years	Lines in groups, %				
	very high resistance (to 10 % of affected plants)	high resistance (10…35 % of affected plants)	mid-resistance (36…60 % of affected plants)	low-resistance (60…85 % of affected plants)	very low-resistance (85…100 % of affected plants)
2008	92,1	5,8	1,6	0,5	0,0
2009	96,4	3,5	0,08	0,0	0,0

The method of express-estimate of sunflower resistance to false downy mildew's pathogen is conducted without application of soil, that makes the work easier and possible to estimate the large number of specimens. In the laboratory 3500 varietysamples for resistance to 310 and 730 races of false downy mildew's pathogen are evaluated by means of the express-method. The intensive selection on resistant samples resulted in the identification of the lines-restorers of fertility – the bearers of genes Pl_2, Pl_5, Pl_6,

Pl_8. Hybridological analysis permit to distinguish donor abilities of these lines ensuring resistance to this pathogen in 100 % of hybrid combinations. Immune and low-affected varietysamples are also recommended for application as donors of resistance by means of inbred crosses and a further rigid selection on the infections sites.

The amount of the phytopathological estimation of the material, which has passed through previous trials in the breeding nurseries under field artificial sites, is from 100 (soil infection on white rot) to 200 specimens (phomopsis) every year.

According to the results of phytopathological estimation in artificial infections sites the hybrids with group resistance to diseases are released: hybrids Borey and Nominal are resistant to phomopsis and soil infection of white rot, hybrids Taim and Ratibor - to phomopsis and head grey rot's pathogens, as well as, to soil infection of white rot. These hybrids have been sent to State Variety Trial of Ukraine.

There are four scientific-research organizations in the system of Ukrainian Acadamy of Agrarian Sciences involved in breeding sunflower. These are: Plant Production Institute nd. a. V. Ya. Yuriev (the city of Kharkov), Selection-Genetical Institute – National Center of Seed investigation and Viriety-studies (the city of Odessa). Institute for Oil-breeding Crops (Zaporozhie), Lugansk Institute of Agroindustrial Production (Lugansk). The cooperation of the research institutions with the experience of many years in sunflower breeding, highly-qualified staff of executors, scientific approach to the problem give the opportunity to carry out a unique multi-factor experiment for widened ecological trial of hybrid combinations in different soil-climatic conditions of Ukraine. Such trials permit to release the samples with high yield potential in combination with a minimal reaction to unfavorable factors of the environment, including, resistant to main pathogens of sunflower in the zone of a future growing of the hybrids. For this purpose every year in 4 soil-climatic zones of Ukraine variety trial of ca. 400 hybrid combinations, developed with the use of the best national germplasm, is conducted. Contrasting conditions for trials as to resistance to diseases' pathogens promote more objective estimation of perspective hybrid combinations and, finally, the competiveness of Ukrainian hybrids at the market grows.

The hybrids, grain yield of which reliably exceeded that of the standards of corresponding groups of maturity by 0,24-0,39 t/ga during the competitive trial in the institute – 2008-2009 ys, or were at the standard's level, when they were studied under artificial infections sites showed high tolerance to soil infection of white rot and to phomopsis (Table 2). Under natural infection by grey rot these hybrids also had a reliably low degree of disease severity.

Table 2 Level of affection of the best hybrids of sunflower bred in Plant Production Institute nd. a. V. Ya. Yuriev of UAAS, necrotrophic pathogens , 2008-2009 ys.

Hybrid	Phomopsis***		White rot****	Grey rot***	Groups of maturity	Grain yield in competitive trial of the institute, t/ga (± to standards)
	natural site	artificial field site	artificial field site	natural site		
Romans[2]	1,9*	10,2*	94,7*	30,0*	II	3,48** (+0,33)
Ryuric[2]	1,7*	11,5*	89,4*	33,3*	II	3,54** (+0,39)
Yason[1]	1,5*	9,2*	89,9*	25,0*	II	3,53** (+0,38)
St Oskil	***2,5***	***17,5***	***92,0****	***30,0****	***II***	***3,15***
Queen[1]	1,3*	7,3*	91,0*	28,4*	III	3,70 (+0,70)
Borey[3]	1,2*	12,4*	96,2*	25,0*	III	3,87** (+0,24)
Sait[3]	1,0*	11,3*	93,1*	30,3*	III	3,64 (+0,01)
Dariy[1]	***1,5****	***6,7****	***93,3****	***25,5****	***III***	***3,63***
Average per trial	***3,5***	***15,8***	***85,1***	***36,9***		
$LSD_{0,05}$	1,1	3,1	4,9	2,3		
$HCP_{0,05}$ compared in pairs						0,23

Notes:

1) * – reliable deviation from average value in trial at 5% of significant level;
2) ** – reliable deviation from standards at 5% of significant level ;
2) *** – disease intensity, %;
3) **** – the number of healthy sprouts, %;

4) [1] – the hybrid is in state Variety List of Russian Federation since 2010;
5) [2] – the hybrid has being in State Variety List of Russian Federation since 2008;
6) [3] – the hybrid has being in State Variety List of Russian Federation since 2009.

A number of sunflower hybrids and varieties highly-productive with group resistance to main diseases' pathogens has been developed by the researchers of the laboratory for breeding and genetics of sunflower and the immunologists of Plant Production Institute nd. a. V. Ya. Yuriev of UAAS during the 20 year period since 1986. For 2010 18 hybrids and 1 variety of the institute selection have been registered in Ukrainian Plant Variety List. The hybrids – Kharkovskiy 49, Zorro, Dariy, Yason, Queen are listed in State Plant Variety Register of Russian Federation; the hybrids – Svitoch and Dariy – in State Variety Register of Belarus.

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Reaction of Iranian sunflower hybrid varieties to downy mildew, *Plasmopara halstedii*

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ABSTRACT

Sunflower downy mildew caused by *Plasmopara halstedii*, is one of the most important diseases in most areas of the crop production areas. The use of resistant varieties and hybrids is an effective method to avoid its damage. Based on existence of a dominant race of the pathogen in sunflower planting areas, 7 new Iranian sunflower hybrids were evaluated to the disease under controlled conditions. Employing whole seedling immersion method with some modifications, three-day-old seedlings were immersed in zoosporangium suspension to be inoculated and then incubated at greenhouse for two weeks. Six qualitative disease characteristics including damping-off, sporulation on cotyledons, sporulation on leaves and cotyledons, stunt, leaf mosaic or chlorosis and deformation were recorded and converted to quantitative means based on their importance in disease development. Finally the average of the means as disease severity index (DSI) was used for evaluations. The all tested hybrids showed significantly resistance against the downy mildew. Furthermore, their related restorer lines have been tested and demonstrated resistance reaction in the experiments.

Key words: sunflower – varieties – downy mildew – resistance – disease severity index

INTRODUCTION

Downy mildew of sunflower caused by *Plasmopara halstedii* has been reported from many countries around the world (Anonymous 1988; Kolt 1985). The disease at first time was reported by Leppik (1962). Then Minassian (1967) and Sharif (1971) found it from west Azerbaijan in Iran. It is considered to be one of the important diseases of this crop in Iran. Incidences more than more than 50% infected plants have been reported during favorable environmental conditions for the disease (Rahmanpour et al. 2000a). Most of the cropping areas of the host show infection including western, northern, and east Northern provinces (Alizadeh and Rahmanpour 2005). The diseased plants with systemic symptoms of infection have the least yield and mostly produce infested seeds. Occurrence of new physiological races of the pathogen encouraged scientists to seek methods for determining resistant resources to the disease. The method whole seedling immersion in zoosporangia suspension was introduced for this purpose (Viranyi 1978) which is used by most of the scientists (Gulya et al. 1991; Mouzeyar et al. 1992). Majority of the sunflower downy mildew researchers consider sporulation on first leaves and cotyledons as susceptibility and the lack of this symptom is categorized as resistance (Bartha et al. 1981; Gulya et al. 1991; Mouzeyar et al. 1992; Viranyi and Gulya 1995). Viranyi et al. (1978; 1980; 1981; 1985) evaluating systemic infection to the disease and comparing susceptible and resistant ones, studied symptoms sporulation on cotyledons, sporulation on leaves, damping-off, hypocotyls' lesions, sporulation on hypocotyls which do not show infection apparently, reduction of root growth, and histological studies. In contrast, Rashid (1993) considered lack of sporulation on cotyledons or leaves as resistance and categorized sunflower varieties and hybrids into five groups resistant, semi-resistant, semi-susceptible, susceptible, and highly susceptible based on percentage of resistant plants.

Cultured area of sunflower in Iran had reached to 100 thousands hectares at 1992 and from this date it was decreased year by year because of several problems. Downy mildew was one of them which restricted the crop at early spring cropping systems. Restrictions on agricultural areas make brilliant the importance of protecting sunflower from biological disorders. Considering long term existence of downy mildew causal agent in contaminated soils, and accessibility of resistant sources to the disease would encourage the breeders to release suitable varieties which can overcome the problem. In this study, using representative isolate of *Plasmopara halstedii*, the reaction of newly improved hybrid varieties of sunflower was evaluated under standard checking method at greenhouse conditions.

MATERIALS AND METHODS

At 2008 soil sample was collected from experimental field at research station of Arak, Central Province, Iran. The field had shown systemic symptoms of downy mildew on sunflower (CMS19) last year. The collected soil was potted into plastic pots and then seeds of susceptible variety Record were sown in the soil. The seeds were incubated for 1-2 months at mostly wet conditions of soil and also 15±2 °C temperature. Inoculated seedlings showing systemic infection to the disease were used to mass-produce the isolate on Record variety. Whole seedling immersion method was employed at this stage (Gulya et al. 1991).

The method whole seedling immersion (WSI) improved by Gulya et al. (1991) was used with some modifications. Seeds of sunflower hybrids and their restorer lines were surface disinfected by sodium hypochlorite (0.25%) for 15 minutes; and after washing and rinsing with sterilized distilled water, were grown into Petri plates for 72 hours. The seeds were incubated at 22 °C, darkness and high humidity. To provide zoosporangia of *P. halstedii*, the leaves of systemic infected Record plants were harvested and put up-turned on a wet layer of filter paper into the Petri plates. Then the plates were put in germinator with 15 °C temperature, and darkness for 24 hours. The produced zoosporangia on the lower surface of infected leaves were brushed gently into sterilized distilled water to make original suspension. The density of suspension was adjusted to 30 thousands zoosporangia per milliliter using haemocytometer slide model Neubauer. To synchronize the exit of zoospores from zoosporangia, sucrose (1%) was added to the adjusted suspension. Three-day-old seedlings were immersed entirely to be inoculated for 4 hours at 15 °C temperature, and darkness. The inoculated seedlings were transferred to greenhouse and planted in pots (13 cm diameter) containing mixture of sand and perlite (1:1 v/v). During two weeks incubation of the seedlings, conditions 22±2 °C temperature and 16 hours photoperiod were provided. Susceptible variety Record as a positive control for inoculation process was used in the experiments. The incubated seedlings with first pair of leaves (2-3 cm length) were covered with plastic bags to provide darkness and high humidity for 24 hours, and the temperature was lowered to 15 °C for inducing the systemic infected plants to sporulate on cotyledons and leaves.

Nine macroscopic characteristics of infection of sunflower to the downy mildew including damping-off, sporulation on cotyledons and leaves, sporulation on leaves, sporulation on cotyledons, stunt, leaf mosaic or chlorosis, deformation, lesions on hypocotyls or crown, and root reduction were used for evaluation. Based on importance of these characteristics, they were quantified scoring them (table 1). The final score (at most 100) for each plant was calculated by summing of scores. The final score was used as disease severity index (DSI) for evaluating the genotypes. Based on DSI, five groups of reaction type were defined for evaluations (Rahmanpour et al. 2000b) (table 2).

Table 1. Quantified symptoms of infection to sunflower downy mildew caused by *Plasmopara halstedii*

Infection symptoms	Score
Damping-off	100
Sporulation on cotyledons and leaves	60
Sporulation on leaves	50
Sporulation on cotyledons	10
Stunt	15
Mosaic/chlorosis of leaves	10
Deformation	5
Hypocotyls/crown lesions	5
Root reduction	5

Table 2. Reaction groups of sunflower to downy mildew based on disease severity index (DSI)

Reaction type	Disease severity index (DSI)
Resistant	0-20
Semi-resistant	20-30
Semi-susceptible	30-50
Susceptible	50-90
Highly-susceptible	90-100

The evaluated genotypes were included: CMS19*R-N1-72, CMS19*R-217, CMS19*R-1031, CMS51*R-864, CMS1221/1*R-14, SHF81-85, and SHF81-90. Three replications for each hybrid were used and each pot as replication contained five plants. Susceptible variety Record was employed as positive control to show the activity of pathogen during inoculation process.

RESULTS AND DISSCUSION

Sever sporulation on both upper and lower surfaces of cotyledons, and lower surface of leaves which is considered as main symptom of susceptibility, was observed on all plants of susceptible variety Record. In addition, obvious stunt (comprised with non-inoculated Record plants), and patterns showing chlorosis on all or most areas of leaves (indicator for systemic penetration of the pathogen) were common on systemically infected plants. In this investigation it has been called as mosaic and statistically had highly regression with sporulation on cotyledons and leaves (Rahmanpour et al. 2004). Totally, the susceptible control plants demonstrated most symptoms referred as infection.

The results showed that all genotypes were categorized into resistant group. A few plants of hybrids SHF81-85 and SHF81-90 demonstrated limited sporulation on cotyledons which was very low dense comparing highly dense sporulation on cotyledons of susceptible variety Record. This phenomenon shows that in resistant genotypes of sunflower, the pathogen could penetrate to hypocotyls and very limited areas of cotyledons close to hypocotyls. But no lesions on hypocotyls and decrease on growth of root were observed.

Alizadeh and Rahmanpour (2005) identified a race as prevalent race of sunflower downy mildew, *Plasmopara halstedii*, for surveyed areas in Iran. Furthermore, the identified race was physiologically different from worldwide determined races proposed by Gulya et al. (1991). But the use of new introduced differential lines is necessary to make sure ourselves for the presence of different physiological race, proposed by Tourvielle de Labrouhe et al. (2000). Importantly, this race is widespread through the most areas of Iran provinces. By releasing sunflower new hybrids containing resistance to downy mildew, damage of the disease on the crop can be controlled significantly.

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II. SUNFLOWER BREEDING ON RESISTANCE TO BROOMRAPE (PARENTAL MATERIAL, BREEDING METHODS, RESULTS)

Affection of European sunflower differentials by broomrape from Rostov region of the Russian Federation

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ABSTRACT

Broomrape, *Orobanche cumana* Wallr., is malicious obligate parasite of sunflower from the higher floral herbaceous plants, capable to destroy the whole yield. In recent years after two decades of absence of a problem connected with broomrape on fields of the Russian Federation information about affection of resistant varieties and hybrids by the parasite have been received from the Rostov, Volgograd, Stavropol and Krasnodar regions. It is thus affected not only domestic sunflower assortment, but also the best resistant foreign hybrids. Well-known European sunflower differentials (corresponding genes of resistance in brackets): LC1002 (*Or4*), LC 1003 (*Or5*), LC1093 (*Or6*), 16Ax25- resistant to race G (received from Dr. Păcureanu-Joita, Romania), P 96 (*or6, or7*) (received from Dr. Melero-Vara, Spain) and VK 623 (*Or5*) (VNIIMK) have been used for studying virulence of broomrape from 31 populations of Rostov region. Broomrape from the majority of populations with a different degree has affected all specified differentials. It testifies that these populations consist of a mix of races C, D, E, F, G with an admixture of extremely dangerous biotype which overcomes action of resistance genes known nowadays. Broomrape from Konstantinovsky, Belokalitvinsky, Tatsinsky, Egorlyksky districts with high degree has affected a differential 16Ax25 which is resistant to race G in Romania. It testifies to considerable concentration of most virulent biotype there. At the same time there have been revealed areas more satisfactory where races E, F, G have not received yet a wide spreading. So in population from Tselinsky district the broomrape does not overcome even action of gene *Or.4*. A population from Salsky district is weakly virulent. According to our data the broomrape from Belokalitvinsky, Egorliksky, Konstantinovsky, Tatsinsky districts, where the concentration of the most virulent biotype is high, differs by strong developed potential of reproductive function. This allows it to expand quickly an area of spreading and to extend others districts which are in favourable condition now.

Key words: sunflower – differentials – broomrape – population – virulence – race

INTRODUCTION

Broomrape (*Orobanche cumana* Wallr.) has centenary history of parasitism on sunflower in Russia. For this period the malicious obligate parasite concerning the higher floral plants, three times threatened disappearance of sunflower, when its cultivation became unprofitable because of strong affection of plants by broomrape. The joint evolution of a broomrape and the host led to appearance of new races of the parasite, capable to overcome immunity of resistant varieties and hybrids. Last epiphytotic conditions developed in the USSR at the beginning seventies when the biotype of broomrape which appeared for the first time in Moldova, named the Moldavian race, started to affect resistant varieties and has quickly extended over all regions of sunflower cultivation.

However in these years successful development and cultivation of new varieties and hybrids resistant to this race has led to exhaustion of the basic resources of parasite seeds in fields because of their germination in the presence of root exudations both susceptible and resistant genotypes of sunflower. Approximately till the end of 1990s problems with broomrape on sunflower had not arisen in Russia. It was even difficult to find and collect the necessary quantity of this plant-parasite seeds for resistance test of breeding material.

However, in last years from different places of the Rostov, Volgograd, Stavropol and Krasnodar regions there has been received information on a strong affection of sunflower fields by broomrape (Antonova et al., 2009). It is thus affected not only domestic sunflower material, but also the best resistant foreign hybrids. It testifies to the future in all places fourth wave of epiphytotic affection of sunflower crops by broomrape in the Russian Federation.

In the European countries cultivating sunflower new broomrape races, overcoming resistance of gene *Or5* have appeared and have quickly extended much earlier on an extent 90th (Melero-Vara, et al., 2000; Molinero-Ruiz, Melero-Vara, 2004; Fernandez-Escobar et al, 2008). Resistance to them of sunflower wild species has been studied and resistant genotypes have been found out, lines and the hybrids resistant against race F, differentials for races have been developed (Dominguez, 1996; Fernandez-Martinez et al, 2000; Perez-Vich et al., 2002; Pãcureanu-Joita et al., 2004, 2008). Nevertheless *O. cumana* is currently regarded as one of the most important constraints for sunflower production in Southern Europe, the Black Sea region, Ukraine and China (Parker, 1994). For the last fifteen years of effort of scientists and breeders to present genetic sources of resistance to this parasite in sunflower hybrids were accomplished by occurrence of new virulent races which quickly have overcome all known genes of resistance (Fernandez-Martinez et al., 2008).

The purpose of our researches was to determine broomrape virulence from different districts of the Rostov area with application of known European sunflower differentials.

MATERIALS AND METHODS

Broomrape seeds were collected from 31 fields in 14 districts of the Rostov region. Well-known European sunflower differentials (corresponding genes of resistance in brackets): LC1002 (*Or4*), LC 1003 (*Or5*), LC1093 (*Or6*), 16Ax25 resistant to race G (received from Dr. Pãcureanu-Joita, Romania), P 96 (*or6, or7*) (received from Dr. Melero-Vara, Spain) and VK 623 (*Or5*) (VNIIMK) have been used for studying broomrape virulence. Susceptible variety VNIIMK 8883 has been also inoculated.

For differentiation of broomrape races seeds of sunflower differentials were sown in plastic boxes with the size 50x20x20 cm, filled with a soil-sandy mix (3:1), mixed with seeds of a parasite. Seeds of each broomrape population added in box from calculation of 200 mg on 1 kg of a soil mix. Plants were grown up in the chamber of an artificial climate at the 16-hours the photoperiod and temperature 22-25^{O}C. Having watered carried out at drying of the top layer of soil. Through 30 days after seedlings appearance plants were dug out and roots were washed with water. Quantity of broomrape individuals (healthy tubercles and stems) were counted up. Average broomrape individuals on one affected plant were calculated on five plants of everyone differentials.

RESULTS AND DISCUSSION

In table 1 data on virulence of six broomrape populations from different districts of the Belokalitvinsky district located in the central part of the Rostov region (fig.1, № 3) are presented. The given tables testify that broomrape from this area overcomes action of genes *Or4, Or5, Or6* and joint action - *or6 or7*. With high degree it has appeared affected differential 16Ax25, resistant against race G in Romania. The insufficient quantity of this differential seeds has not allowed using it for an tests of all populations from this area. Nevertheless, the considerable share of high virulent biotype in one population of that district characterizes it as especially unsuccessful.

Table 1. Affection degree* of sunflower differentials by broomrape from different populations of Belokalitvinskyi district of Rostov region, 2009.

Field	Farm	Hybrid, variety, on which broomrape was collected	VNIIMK 8883	LC1002 **(D)**** *Or4*	LC1003 **(E)** *Or5*	BK623 **(E)** *Or5*	LC1093 **(F)** *Or6*	P 96 **(F)** *or6, or7*	16Ax25 (G) ?
1	OAO Drujba	Garant	72	76	26	30	55	17	-
2	OAO Drujba	Signal	148	75	40	49	38	39	-
3	OAO Drujba	Garant	87	20	58	90	65	39	-
4	Nekhrest and K	Master	68	18	21	13	12	15	36
5	Saturn	PR64A83	106	78	38	35	19	12	-
6	Smetwinovka	Flagman	79	32	34	22	45	19	-

*Affection degree – average of healthy broomrape individuals (tubercles and stems) on 1 affected plant in 30 days after germination (repetated on 5 plants);

* * In brackets it is specified, to what race's differential is resistant

Similarly Belokalitvinsky broomrape from Konstantinovsky, Tatsinsky, Egorlyksky districts overcome action of genes *Or4*, *Or5*, *Or6* and joint action - *or6 or7* and has affected also a differential 16Ax25 with high degree (tab. 2). In the majority of other areas the broomrape biotype, overcoming resistance of 16Ax25, is also found out, though differential affection degree is low for the present. Aksaysky and Morozovsky areas are notable for the higher concentration of race G.

№	Districts of broomrape collection	VNIIMK8883 susceptible	LC1002A **(D)**** *Or4*	LC1003A **(E)**** *Or5*	BK 623 **(E)**** *Or5*	LC1093A **(F)**** *Or6*	P 96 **(F)**** *or6, or7*	16Ax25 **(G)**** **?**
1	Azovsky	36	42	18	27	21	3	-
2	Aksaysky	76	57	15	21	36	4	-
3	Belokalitvinsky	68	18	21	13	12	15	**36**
4	Egorlyksky	82	18	13	4	3	1	**34**
5	Konstantinovsky	119	57	36	23	15	7	**31**
6	Kuybyshevsky	97	10	13	4	4	1	**4**
7	Millerovsky	86	29	34	3	12	1	**6**
8	Morozovsky	118	94	34	21	52	6	**3**
9	Rodionovonesvetaisky	76	6	23	8	1	2	**6**
10	Salsky	72	0	0	1	0	7	**0**
11	Tatsinsky	82	76	35	43	21	12	**3 5**
12	Tselinsky	55	0	0	0	2	0	**0**
13	Tsimlyansky	66	22	9	16	2	3	**8**
14	Sholokhovsky	85	3	0	1	1	0	**2**

*Affection degree – average of healthy broomrape individuals (tubercles and stems) on one affected plant in 30 days after germination (repetition: 5 plants);
* * In brackets it is specified, to which race differential is resistant

Table 2. Affection degree* of sunflower differentials by broomrape from different areas of Rostov region, 2008-2009 (data on the most virulent populations are shown)

At the same time there are areas more satisfactory where races E, F, G have not yet wide spreaded. So in population from Tselinsky district broomrape does not overcome even action of gene *Or4*. A population from Salsky district is also weakly virulent. Though P 96 already had degree of affection 7, action of genes *Or4*, *Or5*, *Or6* (tab. 2) has not been overcome. Also is weakly virulent the broomrape from Sholohovsky district.

According to our data (not published) the broomrape from Belokalitvinsky, Egorlyksky, Konstantinovsky, Tatsinsky districts, where the concentration of the most virulent biotype is high, differ by strong developed potential of reproductive function. This allows it to expand quickly an area of spreading and to extend over other districts which are in satisfactory condition now. Districts Aksaysky and Egorlyksky border on Krasnodar region's territory (fig. 1). Therefore it is necessary to expect that in the nearest future border districts of Krasnodar region also will be occupied by high virulent broomrape.

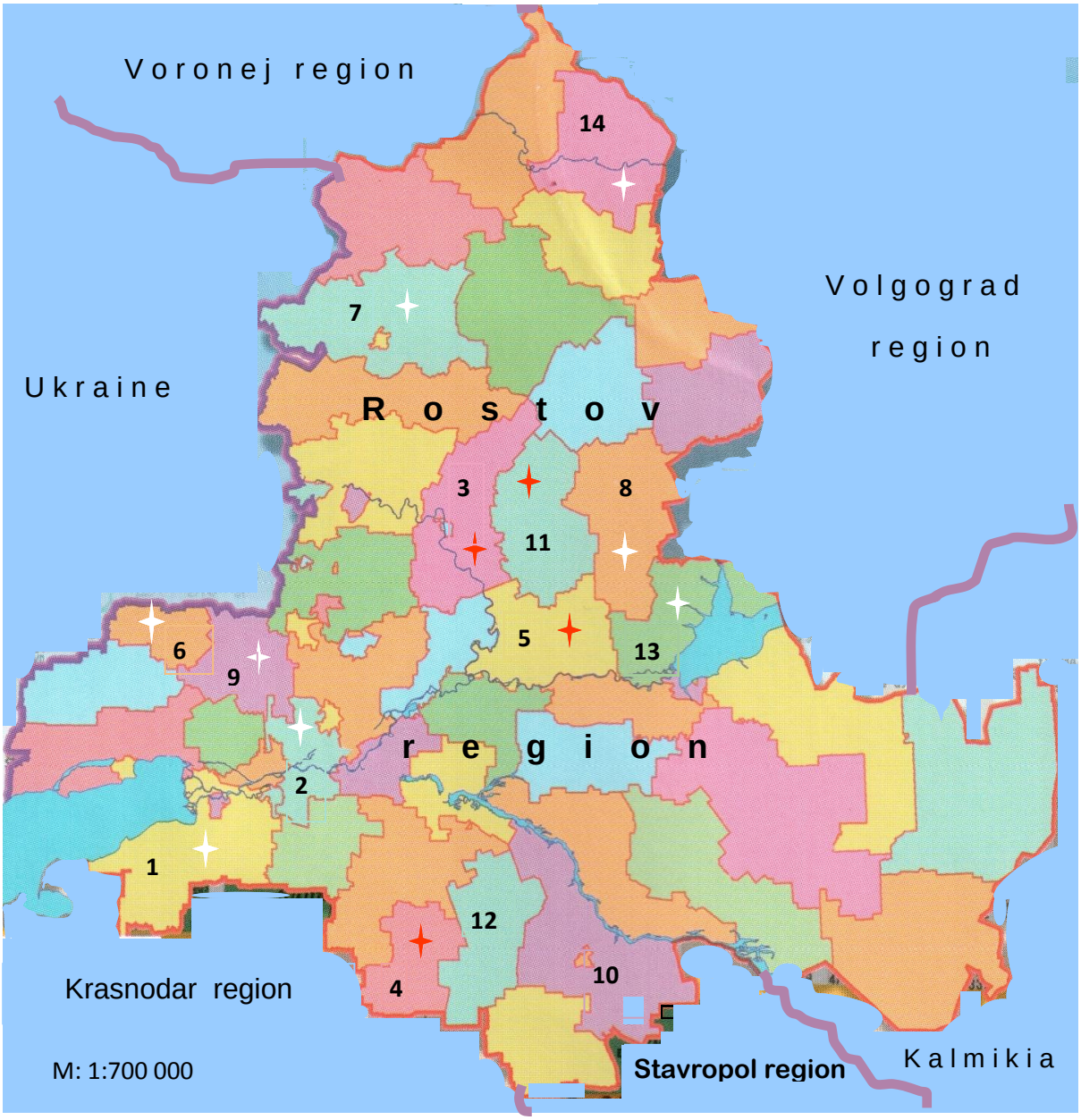

Fig. 1. Presence ( )* of the most virulent *Orobanche cumana* Wallr. in districts of the Rostov region:
1. Azovsky, 2. Aksaisky, 3. Belokalitwinsky, 4. Egorliksky, 5. Konstantinovsky, 6. Kuybyshevsky,
7. Millerovsky, 8. Morozovsky, 9. Rodionovonesvetaysky, 10. Salsky, 11. Tatsinsky, 12. Tselinsky,
13. Tsimlyansky, 14. Sholohovsky;
* -( ) – the red symbol marks areas with high concentration the most virulent broomrape, overcoming resistance of sunflower differential 16Ax25 to race G.

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Effect of imidazolinones on broomrape tubercles in sunflower

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ABSTRACT

The negative influence of imidazolinones on broomrape tubercles on the roots of imi-resistant sunflower plants has been observed. The sunflower inbred lines of VK508 (without broomrape and herbicide resistance) and RHA426 (broomrape susceptible but herbicide resistant) were used. In 10 days after the Pulsar (imazamox) treatment, the plant height and *Orobanche* tubercles were investigated. The plants of line VK508 were killed with herbicide followed by the level of tubercle damage on the roots of 92%. All treated plants of RHA426 survived and possessed the necrotic tubercles on the roots. The damage rate of the tubercles was 64%. This chemical method is considered to be useful in the control of the broomrape in sunflower.

Key words: imidazolinone tolerance – sunflower line – broomrape tubercle

INTRODUCTION

Imidazolinone herbicides have shown to be efficient in controlling of sunflower broomrape *Orobance cumana* Wallr. [2]. A wild population of *Helianthus annuus* L. with resistant genotypes to imazethapyr was found in a soybean field in Kansas, USA [1]. Introgression of genes affecting herbicide resistance from the original wild population to cultivated sunflower was successful and breeding lines have been developed [7]. Imidazolinone resistance is a semi-dominant trait and controlled by two genes [3]. Imidazolinone resistant commercial hybrids of sunflower are considered to be useful in broomrape control in several countries [4, 5, 6].

MATERIALS AND METHODS

The experiment on estimation of imidazolinone effect on sunflower broomrape was conducted in greenhouse in 2006. The seeds of genetically susceptible to broomrape and imidazolinone susceptible breeding line VK508 and susceptible to broomrape and herbicide resistant line RHA426 were sowed in the box with soil-sand mixture. The broomrape seeds collected in Krasnodar, Rostov and Stavropol regions in 2004 were put into the soil-sand mixture in the ratio of 5 g per 10 kg respectively.

Twenty plants of each line VK508 and RHA426 were sprayed with herbicide Pulsar (imazamox) when the plants had 3 pairs of leaves (V6 stage). On the other hand, 20 plants of RHA426 was not treated (check). Concentration of herbicide in water was 3 ml/l (40 g a.i./ha), i.e. 1X dose. After ten days from herbicide treatment sunflower plants were dug out and broomrape tubercles on the roots were counted and measured.

The tubercles on the roots of plants were classified as yellow (yt) – alive tubercles; dark (dt) – tubercles of broomrape with initial stages of tissue necrosis; necrotic tubercles (nt) – dead tubercles with completely dark colour.

RESULTS AND DISCUSSION

After herbicide treatment the plants of inbred line VK508 were dead and characterized the lower height on 6.9 cm than inbred line RHA426. This accounts for the effect of herbicide on the susceptible line VK508 stopping growth after Pulsar treatment on the V6 stage. On the contrary, plants of herbicide resistant inbred line RHA426 continued to grow (table 1).

Untreated inbred line RHA426 had an acceptable attaching rate of average number of 13 tubercles on the roots per a sunflower plant. Almost all of them (98%) were yellow and healthy. Obviously the necrotic tubercles on the roots of the dead sunflower plants of inbred line VK508 had been attached on early plant development stages, i.e. before Pulsar treatment.

Herbicide treatment of inbred line RHA426 showed more number of dark (56 %) and appearance of necrotic tubercles (6 %) in regard to untreated check (fig. 1).

Table 1. Effect of imazamox on the broomrape tubercles on sunflower plants after 10 days of herbicide treatment

Inbred line	Height of plant, cm	Mean number of broomrape tubercles per a plant (%)			
		total	yellow	dark	necrotic
VK508 treated	10.2	4.0	0.3 (8)	1.1 (27)	2.6 (65)
RHA426 treated	17.1	11.0	4.0 (36)	6.3 (56)	0.7 (6)
RHA426 untreated	17.1	13.0	12.7 (98)	0.3 (2)	0.0 (0)
LSD_{05}	2.8	2.8	2.5	1.5	0.8

Fig. 1. Effect of imazamox on the broomrape affection of the line RHA426 (left – treated; right – untreated check)

Moreover yellow broomrape tubercles observed on the roots of treated and untreated sunflower plants of RHA426 differed significantly (table 2). Herbicide treatment leaded to above three times reduction of the size (width) of the tubercles from 1.9 to 0.6 mm.

Table 2. Size and total number of broomrape tubercles observed on the roots of sunflower plants

Inbred line	Average width of tubercles, mm			Total number of tubercles		
	yt	dt	nt	yt	dt	nt
VK508, treated	0.8	0.9	0.7	7	22	53
RHA426, treated	0.6	1.2	1.7	80	127	14
RHA426, untreated	1.9	1.0	0.0	254	6	0
LSD_{05}	0.3	0.4	0.3			

Affection percentage of broomrape on the roots of sunflower plants was calculated as a ratio of the number both of dark and necrotic tubercles to the total number of broomrape tubercles on the roots of each inbred line. That was 92% for dead line VK508, 2% for untreated RHA426 as natural level of lethality of broomrape tubercles and 64% for herbicide treatment RHA426 (fig. 2). The question is whether 36% of small alive tubercles on the roots of RHA426 treated were attached later the date of treatment (low herbicide dose) or these tubercles possessed any kind of tolerance to imazamox (new races of broomrape).

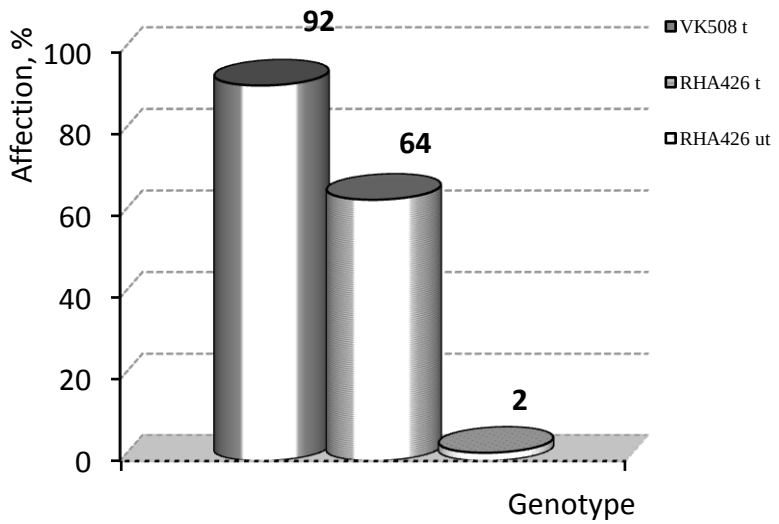

Fig. 2. Affection of broomrape tubercles on the sunflower roots by imazamox
(t – treated with herbicide; ut – untreated)

Therefore, the fact of negative influence of imazamox on broomrape tubercles after treatment of the plants with herbicide Pulsar of genetically broomrape susceptible and imidazolinone resistant sunflower line RHA426 has been confirmed in laboratory conditions. This phenomenon supports the idea of chemical control of broomrape caused damage in sunflower in the south regions of Russia.

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Assessment of quality of new Rf inbred lines resistant to broomrape race E (*Orobanche cumana* Wallr.) developed from *H. deserticola* by interspecific hybridization

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ABSTRACT

Broomrape (*Orobanche cumana* Wallr.) presents a serious problem in sunflower production in a number of countries. The population of broomrape has been stable in Serbia for the longest period of time, but the racial composition has changed in recent years, with race E being predominant in the regions of north Bačka and Banat. Cultivated sunflower is genetically narrow and deficient in many desirable genes. Sources of resistance genes to broomrape can be found in a number of wild sunflower species. New 7 Rf inbred lines (RHA-D-1, RHA-D-2, RHA-D-5, RHA-D-6, RHA-D-7, RHA-D-8, RHA-D-9) were produced from interspecies population originating from *H. deserticola* (DES-1474-1) in IFVC. The inbreeding of the selected plants from interspecies populations started in 1995 (S_1). The first screening of sunflower Rf lines for broomrape resistance was done in a glasshouse in 1999 (S_5). The seed from the resistant plants was tested in infested fields, in the area of S. Miletić and B. Topola during 2000 (S_6). Selection of resistant plants was checked from 2001 (S_7) to 2003 (S_9). The same procedure was conducted in Pačir from 2004 (S_{10}) to 2008 (S_{14}) and in area in Lipar 2009 (S_{15}). Experimental hybrids were produced by crossing new Rf lines developed from interspecies population (DES-1474-1) resistant to broomrape race E with cms female lines susceptible to broomrape. The resistance of new 28 experimental hybrids to broomrape was checked on locations in Serbia (Pačir 2006, 2007, 2008) and at the location (Lipar 2009) with three controls: hybrid Bačvanin, resistant to race E, hybrid NS-H-111 resistant to race A, B, C, D) susceptible to E race and line AD-66 susceptible to all broomrape races. The resistance of the same hybrids was also tested at the location Romania (Baragan, Braila) in 2008. All examined hybrids were resistant to broomrape race E on all locations.

Key words: sunflower – interspecific hybridization – resistance

INTRODUCTION

Broomrape is one of the largest problems in sunflower production, it reduces sunflower seed yield and negatively affects other sunflower traits (Demirci and Kaya, 2009). Different pathogenic races of *Orobanche cumana* Wallr. are known to exist in various regions of Europe and in the Southeastern Mediterranean where the climate is favorable for this parasite Virány F. (2008). The virulence of the parasite populations of broomrape has changed over years, slowly at first, then rapidly in Eastern Europe, Turkey and Spain. Parasite is becoming more and more dangerous for the sunflower crop (Pacureanu et al., 2008). Broomrape resistance is poorly understood and new races of the parasite are evolving rapidly and overcoming the resistance of newly introduced sunflower hybrids (Virány F., 2008). It can significantly reduce seed and oil yield, weight of 1000 seeds, seed oil content, plant height and head diameter in sunflower production (Kaya et al., 2004). Breeding strategies for incorporating broomrape resistance into sunflower commercial hybrids have been nearly exclusively based on single race-specific dominant genes, which are considered by seed companies as an ideal source of resistance for single-cross hybrid breeding, as they only need to be incorporated into one of the parents (Fernández-Martínez et al., 2008). The resistance to races is controlled by single dominant genes (Or). With the increase of the sunflower covered areas in the world, new races of broomrape have also developed. Until few years ago among the sunflower broomrape populations only 5 physiological races (A, B, C, D and E) and five sunflower dominant genes (Or_1, Or_2, Or_3, Or_4 and Or_5) conferring the resistance towards broomrape races were known. Recently there has been a sudden change in race composition. New races have appeared: race F in Romania, Bulgaria, Spain and Turkey. The new broomrape races can not be controlled by Or_5 gene (race E). The changes in the broomrape population and the appearance of new races represent a crucial point in sunflower breeding, forcing the breeders to continually test the breeding material against new broomrape races while creating differential lines (Hladni et al., 2009). Sources of resistance to the

recent virulent races E and F have been located in cultivated germplasm (Gulya et al., 1994; Fernández-Martínez et al. (2000), in contrast, a high level of resistance to races E, F and G has been found in the evaluation of wild *Helianthus* species, particularly perennials (Škorić, 1988; Ruso et al., 1996; Fernández-Martínez et al., 2000), revealing that wild *Helianthus* species constitute a major pool of genes for resistance to the new virulent races of broomrape. Different ways for controlling parasite attacks were tested (different methods of soil cultivation, herbicides use, biological agents, etc.), which gave no viable practical results. Till now the broomrape problem was mainly solved by genetic means, by finding new resistance sources and developing new sunflower material resistant to the new parasite races (Christov et al., 2009). Epiphytotic occurrence of broomrape was observed in Serbia for the first time in 1951. During a longer period of time the broomrape population in Serbia was stable. Race B is dominant in the South of the Vojvodina Province and race E in the North (Dedić et al., 2009). Race E has expanded to the Bačka and Banat area and is slowly starting to become a big problem in sunflower production. In 2008, infected areas were found in Bor County (two sunflower plots) near the state border with Romania and Bulgaria (Dedić et al., 2009; Maširević et al., 2009). Continual monitoring of the broomrape population in Serbia is very important due to changes in race composition and evolution of new more virulent races in neighbouring countries and also because climate changes are favorable for expansion of *Orobanche* species to lage areas Maširević et al. (2009). The sunflower breeding program at the Institute of Field and Vegetable Crops has been directed towards creating lines and hybrids which will be resistant to new races of broomrape used interspecies hybridization. In this investigation several interspecies populations with *H. deserticola* were initially used for the production of Rf male inbred lines resistant to drought. When creating hybrids resistant to drought and high temperatures it is important to use wild species genus *Helianthus*, which inhabit desert and semi-desert areas (Fick and Miller, 1997; Škorić, 2009). Several agronomicaly important traits of new Rf inbred lines and their hybrids were evaluated and are discussed in the paper. Unexpectedly the resistance to broomrape race E was consistent on all examined locations in Serbia (Pačir 2006, 2007, 2008 and Lipar 2009) and at the location Romania (Baragan, Braila 2008). This result indicates that some inbred lines developed from interspecies populations originating from *H. deserticola* are resistant to broomrape and can be used for the production of new resistant sunflower hybrids.

MATERIALS AND METHODS

New Rf inbred lines (RHA-D-1, RHA-D-2, RHA-D-5, RHA-D-6, RHA-D-7, RHA-D-8, RHA-D-9) were produced from interspecies population originating from *H. deserticola* in Institute of Field and Vegetable Crops (IFVC). Initially the plants were selected from the interspecies population DES-1474-1, 2, 3 provided by Dr Gerald Seiler (USDA-ARS, Fargo ND, USA) originating from *H. deserticola.* The inbreeding of selected plants from interspecies populations started in 1995 (S_1). The first screening of sunflower Rf lines for broomrape resistance was done in glasshouse in 1999 (S_5). The seed from the resistant plants was tested in infested fields, in the area of S. Miletić and B. Topola during 2000 (S_6). Selection of resistant plants was continued in 2001 (S_7), 2002 (S_8) and 2003 (S_9). The same procedure was conducted in Pačir from 2004 (S_{10}) to 2008 (S_{14}) and Lipar 2009 (S_{15}) which resulted with 7 resistant restorer inbred lines. In all locations lines were sown in 3 rows (12 plants per row), of which only the plants the middle one were evaluated. In order to measure the morphophysiological traits of resistant lines the experiment was set at an experimental field of the IFVC at Rimski Šančevi, in a randomized complete block system with three replications during 2008/2009. The basic sample for the analysis of the examined trait contained 30 plants (10 plants per replication) sampled from the middle row of each block. The plant height (PH) and head diameter (HD) were measured (cm) in the field at the stage of physiological maturity. The analysis of oil content (SOC) in seed was carried out nondestructively on a nuclear magnetic resonance (NMR) analyzer. Seed protein content (SPC) is determined by Kjeldahl method. Both analyses were at the chemical laboratory of the Institute's Oil Crops Department. On a random sample of completely pure and air dried seed the weight of 1000 seeds (M1000S) was determined (g). The resistance of 28 new experimental hybrids to broomrape was tested on locations in Serbia (Pačir) during three years (2006, 2007 and 2008) and (Lipar) in 2009. In Romania new experimental hybrids to broomrape was tested in (Baragan, Braila) during 2008. These hybrids were produced by crossing female inbred lines (Ha-26PR-A, PH-BC_2-92-A, Ha-98-A and HA-1200A) resistant to race D but susceptible to race E and new Rf inbred lines (RHA-D-1, RHA-D-2, RHA-D-5, RHA-D-6, RHA-D-7, RHA-D-8, RHA-D-9), developed from interspecies population originating from *H. deserticola*, and resistant to broomrape race E. Control sunflower genotypes used in Serbia were the line AD-66 susceptible to all broomrape race, hybrid NS-H-111 resistant to race (A, B, C, D) susceptible to E race and the hybrid Bačvanin, which

is resistant to the broomrape race E (contains gene Or_5). The basic sample for the analysis of the examined trait contained 40 plants (20 plants per replication) sampled from the middle row of each block. In the small plot trials 28, NS-experimental hybrid was set up in 2008 and 2009 on the location of Rimski Šančevi, Serbia. In a randomized complete block system with three replications, with he basic field size of 28 m^2 (4 rows). The hybrids were evaluated for PH, HD, SY, SOC and OY. The seed yield (SY) was normalized to 11% of seed moisture content. Oil yield (OY) was calculated from the seed yield and oil content. High yield hybrids Baća, Šumadinac resistant to broomrape (race E) and Sremac non-resistant to broomrape were used as standards. The determination of mean values and ANOVA two –factor without replication Hadživuković (1991).

RESULTS AND DISCUSSION

Broomrape is present in Province Vojvodina as the main sunflower production region in Serbia. Main hazardous areas are sunflower fields on the route Subotica - B. Topola, with a tendency of spreading toward Senta and Čantavir, and then to the south. Less intensive attack has also been noticed in Banat around Padej, Itebej and Vršac. In 2008, infected areas were found in Bor County (two sunflower plots) near the state border with Romania and Bulgaria (Figure 1). The continuous problem with broomrape spread asked for continuous research work in the filed, searching for new sunflower materials resistant to the new races. Till now the broomrape problem was mainly solved by genetic means, by finding new resistance sources and developing new sunflower material resistant to the new parasite races. *H. deserticola* can be used for drought tolerance as well as oil concentration and quality improvement (Seiler, 2007). Breeding of sunflower inbred lines originating from interspecies populations DES-1474-1, DES-1474-2 and DES-1474-3, in order to implement drought tolerance, started in 1994 at the IFVC. Apart from the analysis of important agronomic traits new restorer inbred lines were tested for resistance to broomrape. A continued work on the testing of restorer inbred lines was conducted from 1995 to 2003, which resulted in 7 restorer inbred lines originating from interspecies population DES-1474-1, resistant to broomrape race E (Hladni et al., 2009). The resistance of new lines checked on location Pačir from 2004-2008 and location Lipar 2009 with three controls: AD-66 line susceptible to all broomrape races, hybrid NS-H-111 resistant to race A, B, C, D and hybrid Bačvanin-resistant to broomrape race E (Table 1). New Rf lines studied differed significantly in the mean values of all the traits under investigation: PH varied from140cm(RHA-D-6) to 172cm(RHA-D-8), HD ranged from 12.0cm(RHA-D-1) to 14.5cm(RHA-D-9), SOC varied from 38.3%(RHA-D-9) to 46.3%(RHA-D-7), SPC from 18.8%(RHA-D-6) to 22.0%(RHA-D-9) and 1000sm from 41.0g(RHA-D-1) to 58.7g(RHA-D-6) (Table 1). The resistance of 28 new experimental hybrids to broomrape was confirmed on locations in Serbia (Pačir 2006-2008) and location (Lipar 2009) with three controls: hybrid Bačvanin, hybrid NS-H-111 and line AD-66. As the race F is present in Romania, the resistance of the same hybrids was also tested and confirmed there, at the location (Baragan, Braila, 2008) (Table 2). During 2008/2009 on the location of R. Šančevi morphophysiological traits of new experimental sunflower hybrids resistant to broomrape were examined. Significant differences were determined in the mean values for all the traits studied: PH from 165cm(NS-H-12) to 205cm(NS-H-6), HD 19.0cm(NS-H-22) to 23.1cm(NS-H-12), SY from 3.45tha^{-1}(NS-H-22) to 4.19tha^{-1}(NS-H-6), SOC from 46.4%(NS-H-25) to 50.1%(NS-H-16) and OY from 1.61 tha^{-1}(NS-H-22) to 2.09tha^{-1}(NS-H-16). Ten new hybrids resistant to broomrape were significantly better mean values SOC in relation to SOC achieved with the control NS-H-111(48.4%) which were significantly better than all for standard hybrids (Table 2). On the basis of two year results for the agronomicaly most important traits such are SY, SOC and OY gained on the location of R. Šančevi during 2008/09, new five hybrids resistant to broomrape NS-H-6(SY 4.19tha^{-1}; SOC 48.2%; OY 2.02tha^{-1}), NS-H-16(SY 4.17tha^{-1}; SOC 50.1%; OY 2.09tha^{-1}), NS-H-19(SY 4.15tha^{-1}; SOC 49.1%; OY 2.04tha^{-1}), NS-H-20(SY 4.13tha^{-1}; SOC 48.8%; OY 2.01tha^{-1}), NS-H-18(SY 4.10tha^{-1}; SOC 48.4%; OY 1.99tha^{-1}) had significantly better SY in relation to the best standard, Sremac (SY 4.01 tha^{-1}) and SOC and OY in relation to the best standard Šumadinac (SOC 47.9%; OY 1.82tha^{-1}), table 2.

Interspecies hybridization or the discovery of desirable genes in the wild species of the genus *Helianthus* and their incorporation into cultivated sunflower genotypes, which is a valuable source of resistance genes, holds a special place in sunflower breeding and makes room for the discovery of the resistances towards different broomrape races which has been shown in this paper. New sources of resistance to broomrape have often been searched for in wild species, with focus on *H. tuberosus* (Jan et al., 2002). Most of the NS hybrids resistant to broomrape, produced so far, were based on interspecies populations with *H. tuberosus*, among them hybrid Bačvanin, used as a control in our research. Results on the resistance of other sunflower wild populations are scarce. Seiler (1994) showed that interspecific lines

derived from *H. anomalus*, *H. argophyllus* and *H. deserticola* showed some level of resistance against broomrape race SE-192. Ruso et al. (1996) defined only three interspecies populations (ANO-1509-2-2, ARG-420-1-2 and DES-1474-1-2 derived from *H. anomalus*, *H. argophyllus* and *H. deserticola* respectively) that were partially resistant to broomrape race SE-193. It is known that among each wild species there is a great variability in populations that mutually differ when it comes to the values of the majority of qualities and traits (Seiler and Gulya, 2004). Here we show that Rf inbred lines developed from interspecies population with *H. deserticola* - DES-1474-1, are resistant to broomrape race E and possibly F, and indicate that this population can be used for the production of new resistant sunflower hybrids.

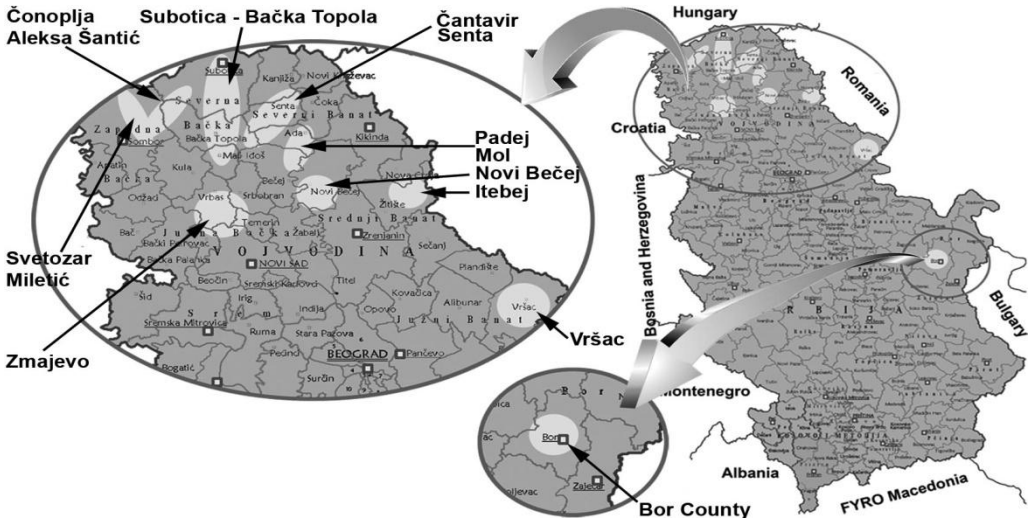

Fig. 1. The main areas of broomrape infestation in the sunflower crop in Serbia

Table 1. Rf inbred lines resistant to broomrape race E created from interspecies populations originating from *H. deserticola* and their values of plant height, head diameter, seed oil content, seed protein content and mass of 1000 seed in 2008/2009

	Population DES-1474-1	Pačir 04-08	Lipar 09	Traits	PH	HD	SOC	SPC	1000 sm
	Rf inbred lines	S_{10}-S_{14}	S_{15}		cm	cm	%	%	g
1	RHA-D-1	R	R		150	12.0	40.5	20.1	41.0
2	RHA-D-2	R	R		149	12.5	39.2	20.7	47.2
3	RHA-D-5	R	R		160	12.8	40.1	21.2	48.5
4	RHA-D-6	R	R		140	13.5	44.2	18.8	58.7
5	RHA-D-7	R	R		144	12.7	46.3	20.4	50.5
6	RHA-D-8	R	R		172	14.2	44.1	21.1	51.5
7	RHA-D-9	R	R		152	14.5	38.3	22.0	43.8
control	AD-66	S	S	LSD 0.05	8	0.3	0.9	0.8	1.5
control	NS-H-111	S	S	LSD 0.01	13	0.5	1.4	1.2	2.4
control	Bačvanin	R	R						

(R) + broomrape absent; (S) - broomrape present on every plant; PH-plant height, HD-head diameter, SOC-seed oil content,
SPC-seed protein content, 1000sm-mass of 1000 seed

Table 2. Testing of NS sunflower hybrids in field conditions on parcels infected by broomrape on the locations of Pačir (P) from 2006-2008, Lipar (L) from 2009 and Braila (B) from 2008 and mean values of morphophysiological traits of the hybrids tested during 2008/2009 on the location of Rimski Šančevi (RŠ)

	F_1 hybrids			P	L	B		RŠ				
		Cms	Rf				Traits	PH	HD	SOC	SY	OY
				06-08	09	08		cm	cm	%	tha^{-1}	tha^{-1}
1	NS-H-1	Ha-26PR-A	RHA-D-1	R	R	R		179	20.5	47.1	3.71	1.75
2	NS-H-2		RHA-D-2	R	R	R		177	20.5	47.1	3.81	1.79
3	NS-H-3		RHA-D-5	R	R	R		195	21.5	48.1	3.64	1.75
4	NS-H-4		RHA-D-6	R	R	R		171	21.0	47.4	3.73	1.76
5	NS-H-5		RHA-D-7	R	R	R		169	22.0	48.1	4.00	1.92
6	NS-H-6		RHA-D-8	R	R	R		205	23.0	48.2	4.19	2.02
7	NS-H-7		RHA-D-9	R	R	R		185	21.0	48.4	4.04	1.96
8	NS-H-8	PH-BC_2-92-A	RHA-D-1	R	R	R		177	20.0	47.3	3.71	1.76
9	NS-H-9		RHA-D-2	R	R	R		175	20.0	48.4	3.76	1.82
10	NS-H-10		RHA-D-5	R	R	R		185	21.0	48.2	3.73	1.80
11	NS-H-11		RHA-D-6	R	R	R		175	20.0	47.6	3.55	1.69
12	NS-H-12		RHA-D-7	R	R	R		165	23.1	48.2	3.56	1.71
13	NS-H-13		RHA-D-8	R	R	R		200	21	48.4	3.96	1.91
14	NS-H-14		RHA-D-9	R	R	R		175	21.5	48.5	3.96	1.92
15	NS-H-15	Ha-98-A	RHA-D-1	R	R	R		193	21.0	49.6	3.98	1.97
16	NS-H-16		RHA-D-2	R	R	R		192	22.0	50.1	4.17	2.09
17	NS-H-17		RHA-D-5	R	R	R		193	22.0	49.0	3.53	1.73
18	NS-H-18		RHA-D-6	R	R	R		181	22.0	48.4	4.10	1.99
19	NS-H-19		RHA-D-7	R	R	R		183	23.0	49.1	4.15	2.04
20	NS-H-20		RHA-D-8	R	R	R		200	23.0	48.8	4.13	2.01
21	NS-H-21		RHA-D-9	R	R	R		195	21.0	48.8	3.99	1.95
22	NS-H-22	HA-1200A	RHA-D-1	R	R	R		191	19.0	46.6	3.45	1.61
23	NS-H-23		RHA-D-2	R	R	R		190	20.0	46.9	3.55	1.66
24	NS-H-24		RHA-D-5	R	R	R		193	19.5	46.9	3.59	1.68
25	NS-H-25		RHA-D-6	R	R	R		183	20.0	46.4	3.70	1.72
23	NS-H-26		RHA-D-7	R	R	R		181	20.5	48.1	3.90	1.87
27	NS-H-27		RHA-D-8	R	R	R		205	22.0	48.0	3.88	1.86
28	NS-H-28		RHA-D-9	R	R	R		193	19.0	47.2	3.95	1.86
							standard					
							Baća	185	22.5	47.7	3.63	1.73
	control	AD-66		S	S		Šumadinac	170	23.5	47.9	3.81	1.82
							Sremac	179	24.0	44.9	4.01	1.80
	control	NS-H-111		S	S			185	23.0	48.4	3.52	1.70
	control	Bačvanin		R	R			180	23.0	48.0	3.65	1.75
	LSD	0.05						1.5	0.8	1.5	0.46	0.22
	LSD	0.01						2.0	1.0	1.9	0.62	0.30

(R) + broomrape absent; (S) - broomrape present on every plant; PH-plant height, HD-head diameter, SOC-seed oil content, SY-seed yield; OY-oil yield.

CONCLUSION

Wild *Helianthus* species constitute the major reservoir of genes offering resistance to new virulent broomrape races. New NS hybrids resistant to broomrape race E developed from interspecies population with *H. deserticola* have achieved higher seed and oil yield in relation to standard hybrids resistant to race E broomrape Baća, Šumadinac and Bačvanin. It is expected that after recognition by the Serbian Supervising and Testing Institute these hybrids will find their place in the production on the broomrape infected fields.

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Broomrape control and phytotoxicity of imidazolinone herbicide in IMI sunflower genotypes and influence on seed yield

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ABSTRACT

Imidazolinone herbicides are one of the most feasible method for broomrape control, especially in the intensive agriculture. In this trial we evaluated resistance of seven different sunflower genotypes of F_1 and F_2 generation and five commercial hybrids to imidazolinone herbicides and also their effect on broomrape control. The experiment was conducted in field conditions in Svetozar Miletić locality (North Serbia), at naturally highly infested plot. Race E was determinated in this region ten years ago for the first time in Serbia. The analysis of population, which was done by set of differential lines, showed that broomrape in that locality still belong to race E of the parasite.

Sunflower was treated with imidazolinone herbicide (Pulsar-40) with 1.2 l/ha (at concentration rate 0.25%) at three leaf stage. Control were the same non-treated genotypes and commercial hybrid NS-H-111 which is susceptible to broomrape and IMI herbicides. Evaluation of herbicide effect was done 14 days after application when sunflower plants were in the beginning of budding stage. The reaction of sunflower genotypes to applied herbicide was evaluated according to the reaction of plants. The phytotoxicity of herbicide was expressed by following symptoms: total plant deterioration, severe chlorosis and slightly yellowing. Intensity of phytotoxicity varied depending on the observed genotype. Percentage of dead plants ranged from 13-68%, plants with severe chlorosis and curly leaves ranged from 0-35% and symptoms of slightly chlorosis were observed on up to 20% percentages of evaluated plants. Plants which expressed susceptibility to applied herbicide such as chlorosis and yellowing totally recovered during the time but the phytotoxicity had influence on the yield and quality of seeds. Seed yield of plants with symptoms has been decreased from 43,7 to 90,1% related to control. Although we obtained good results in broomrape control in sunflower by one application of IMI-herbicide, we recommend two split applications of IMI herbicides.

Key words: broomrape – IMI-herbicides – sunflower – resistant genotypes

INTRODUCTION

Broomrapes (*Orobanche* spp) belong to the family *Orobanchaceae*, obligate parasitic flowering plants. Broomrapes (*Orobanche* spp.) are native primarily to the Mediterranean region (i.e. North Africa, the Middle East, and southern Europe), and western Asia, where they cause significant crop damage (Parker and Riches, 1993). With anticipated climatic changes taking the form in higher temperatures and drought in many areas of the world, *Orobanche* species could pose greater threats to agriculture by expanding their ranges farther north in Europe and elsewhere (Mohamed et al. 2006). According to Aćimović (1977) broomrape for the first time was described in Serbia in 1951 Since that period it has been appearing with varying intensity almost every year but since the 1990s broomrape has been causing significant damage in susceptible sunflower hybrids (Maširević and Medić-Pap, 2009). Yield losses depend on intensity of attack and they can range from 5 to 100 % (Maširević, 2001). All broomrape races can be successfully controlled by chemical means as well, namely by growing IMI-resistant hybrids (RIMI) in synchrony with the application of appropriate imidazolinone-based herbicides (Skorić and Jocić, 2005).

MATERIALS AND METHODS

Plant material

In this trial we evaluated resistance of male parental line A-B-IMI-1B, seven different sunflower genotypes of F_1 (AB-ORO-5B x AB-IMI-1B, AB-ORO-11B x AB-IMI-1B, AB-ORO-14B x AB-IMI-1B, AB-ORO-34B x AB-IMI-1, AB-ORO-39B x AB-IMI-1B, AB-ORO-40B x AB-IMI-1B, AB-ORO-43B x AB-IMI-1B) F_2 generation (F_1 5B x 1B, F_1 11B x 1B, F_1 14B x 1B, F_1 34B x 1B, F_1 39B x 1B, F_1 40B x 1B, F_1 43B x 1B), three commercial hybrids (MI-3-911, IMI-3-369 (AKA Argentina) PARAISO-120CL (NIDERA Argentina)) and two experimental hybrids (ATO301CL x RHA-1R3RF, ATO521CL x RHA-1R3RF) to imidazolinone herbicides and also their effect on broomrape control.

The experiment was conducted in field conditions in Svetozar Miletić locality (North Serbia), at naturally highly infested plot. This area is know as main foci of hazard. Hybrids and F_1 genotypes were

sown in 4 rows and F_2 genotypes were sown in 8 rows (25 plants in row). Differential lines for determination of broomrape races were also sown in the experimental field.

Evaluation of imidazolinone herbicide effect

Sunflower was treated with imidazolinone herbicide (imazamox) (Pulsar-40) with 1.2 l/ha (at concentration rate 0.25%) at six leaves stage (figure 1.). Control were the same non-treated genotypes and commercial hybrid NS-H-111 which is susceptible to broomrape and IMI herbicides. Evaluation of herbicide effect was done 14 days after application when sunflower plants were in the beginning of budding stage. The reaction of sunflower genotypes to applied herbicide was evaluated according to the reaction of plants. Heads of the plants on which were noticed severe phytotoxic effect caused by herbicide were isolated in the purpose of yield measurment. Plants which did not express any symptoms of phytotoxicity were used as a control and their heads were also isolated. Reaction of sunflower genotypes was followed untill the end of vegetation period and finaly two medium rows of every genotype were taken for yield estimation.

Fig. 1. Experomental field (Svetozar Miletic,North Serbia)

Evaluation of intesity of broomrape attack

Observations of broomrape were evaluated as frequency (F), intensity (I) and attacking rate (AR). Frequency is a percent of plants with *Orobanche*. Intensity is the number of *Orobanche* in one infested plant and attak rate is the number of *Orobanche* in one plant in the row.

RESULTS AND DISCUSSION

The analysis of population, which was done by set of differential lines, showed that broomrape in Svetozar Miletić locality still belong to race E of the parasite.

In table 1. we showed only genotypes which were infected with broomrape. None of the experimental IMI resistant hybrids, F_1 and F_2 which were treated with Pulsar were infected with broomrape. Three out of seven F_2 genotypes which were not treated with Pulsar were infected with broomrape, but frequency and attacking rate in these genotypes were low. Frequency range from 1.56-6.63% and attacking rate ranged from 0.0003-0.12 (table 1). These genotypes can be classified as resistant according to Vranceanu *et al.*, 1980 and Maširević, 2002, the plants having 0-10% frequency and 0-1 AR values were accepted as resistant. The control genotype NS-H-111 has 31.25% of infected plants and 1.11 broomrapes per sunflower plant (attack rate) (figure 2.).

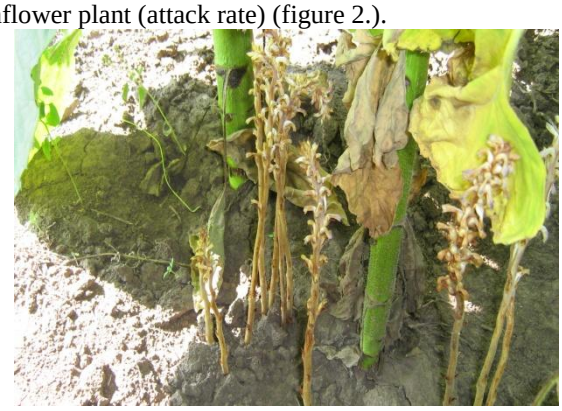

Fig. 2. Broomrape attack in suscetabile NS-H-111

Table 1. Broomrape observation in sunflower F_2 genotypes and control*

Hybrid	Frequency F (%)	Intensity I	Attack rate
F_1 11B X 1B**	2.75	0.25	0.03
F_1 14B X 1B**	1.56	0.5	0.0003
F_1 34B X 1B**	6.63	1.38	0.12
NS-H-111	31.25	14.9	1.11

*in table 1 we show only genotypes which have broomrape attack

** non treated F_2 genotypes

In our trial some sunflower plants treated with Pulsar expessed the symptoms of herbicide phytoxicity. Number of plants with symptoms in male line and F_1 and F_2 genotypes and experimental hybrids are shown in table 2. The phytotoxicity of herbicide was expressed by following symptoms: total plant deterioration, severe chlorosis (figure 3.) and slightly yellowing. Intensity of phytotoxicity varied depending on the observed genotype. According to Massing et al. (2005) the plants with less than 20% imazamox injury were classified as IMI-resistant.

Number of plants which expressed symptoms after herbicide treatment in experimental hybrids, male line and F_1 and F_2 were shown in table 2. Percentage of dead plants ranged from 13-68%, plants with severe chlorosis and curly leaves ranged from 0-35% and symptoms of slightly chlorosis were observed on up to 20% percentage of evaluated plants. Plants which expressed susceptibility to applied herbicide such as chlorosis and yellowing totally recovered during the time but the phytotoxicity had influence on the yield and quality of seeds. The male line A-B-IMI-1B has 99,20% healthy plants after herbicide treatment, so it can be concluded that these line is homozygous in resistance for IMI herbicides. The higest number of dead plants (over 50%) were found in three F_1 lines (AB-ORO-11B x AB-IMI-1B, AB-ORO-34B x AB-IMI-1B and AB-ORO-39B x AB-IMI-1B). Also in these genotypes there were no plants with sligth herbicide injuries and number of plants with severe symptoms were very low. In other four F_1 genotyopes the number of dead plants ranged from 12-32%. It is indicative that only in one F_1 line (AB-ORO-14B x AB-IMI-1B) slight herbicides injuries were recorded. Number of dead plants was uniform in four F_2 genotypes and was about 20% in other two genotypes it was about 14 and in one 28%. Sever injuries of hercides in F_2 genotypes were noticed from 5-35%. According to the obtained results (table 2.) in backcross generation 6 out of 7 genotypes have significant less dead plants than in F1 generation. So, plants in the first backcross generation showed higher degree of resistance.

It is very interesting that in one experimental hybrid IMI-3-396 we have one plant with severe symptoms of herbicide deterioration and in hybrid ATO301CL x RHA-1R3RF beside the a yellow curly sunflower plant there was one dead plant.

Fig. 3. Phytotoxicity symptoms on sunflower plants caused by herbicide treatment

Table 2. Phytotoxicity of herbicide in experimental hybrids, male line and F_1 and F_2 genotypes

Genotype	Number of plants with slight phytotoxicity symptoms (%)	Number of plants with severe phytotoxicity symptoms (yellow curly plants) (%)	Number of dead plants (%)	Number of healthy plants (%)
male line A-B-IMI-1B	0	0,80	0	99,20
AB-ORO-5B x AB-IMI-1B	0	7,69	12,82	79,49
F_1 5B x 1B	3,41	13,64	19,32	63,64
AB-ORO-11B x AB-IMI-1B	0	9,32	55.81	43,88
F_1 11B x 1B	20,75	15,09	28,30	35,85
AB-ORO-14B x AB-IMI-1B	7,31	7,31	31,71	53,66
F_1 14B x 1B	2,20	9,89	14,29	73,63
AB-ORO-34B x AB-IMI-1B	0	9,52	54,76	35,71
F_1 34B x 1B	0	16,47	20,00	63,53
AB-ORO-39B x AB-IMI-1B	0	0	68,29	31,71
F_1 39B x 1B	1,67	18,33	18,33	61,67
AB-ORO-40B x AB-IMI-1B	0	0	28,57	71,43
F_1 40B x 1B	0	35,56	20,00	44,44
AB-ORO-43B x AB-IMI-1B	0	0	25,00	75,00
F_1 43B x 1B	3,70	4,94	14,81	76,54
hybrids				
IMI-3-911	0	0	0	100
IMI-3-396	0	1.14	0	98.86
PARAISO 102CL	0	0	0	100
ATO301CL x RHA-1R3RF	0	1.14	1.14	97.73
ATO521CL x RHA-1R3RF	0	0	0	100

Yield of plants with or without symptoms of herbicide phytotoxicity are shown in table 3. Seed yield of plants with symptoms has been decreased from 43,7 to 100 % related to healthy plants. These results show that IMI herbicide inflences on sunflower plants and cause not only visible injuries but also it cause yield decrease.

Table 3. Yield of plants with or without symptoms of herbicide phytotoxicity

Genopyte	Yield per plant		Yield ratio (%)
	plants with symptoms of herbicide phytotoxicity	plants without symptoms of herbicide phytotoxicity	
F_1 5B x 1B	3.1	7.5	58.7
F_1 11B x 1B	0	11.5	100
F_1 14B x 1B	3.6	40.3	91.1
F_1 34B x 1B	7.5	15.8	52.5
F_1 40 x 1B	14.2	25.2	43.7

CONCLUSION

The analysis of population showed that broomrape in Serbia belongs to race E. Constant monitoring of broomrape population in Serbia is very important due to changes in race composition in neighboring countries. It is also very important to develop control measures for suppression of *Orobanche* in sunflower.

Strategy for broomrape control should be cultivation of resistant sunflower hybrids including IMI resistant hybrids and broomrape tolerant hybrids which suppressed and decreased weed population and epidemics.

In breeding process much less percent of dead plants were obtained in the first backcross generation. These results encourage, because the aim is to obtain broomrape resistant genotypes which are also resistant to the IMI herbicides.

The most of plants with symptoms of herbicide phytotoxicity recover, but the treatment has influence on their yield. As number of such plant is higher, the yield decreases more.

Although we obtained good results in broomrape control in sunflower by one application of IMI-herbicide, we recommend two split applications of IMI herbicides.

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Broomrape (*Orobanche cumana* Wallr.) in sunflower crop in Romania

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ABSTRACT

Broomrape (*Orobanche cumana* Wallr.) is the most important problem in sunflower crop in southearn and southeastern Romania. This leads to considerable losses expressed in sunflower seed yield decrease and in a low quality of the obtained produce.

In the last years, the parasite has developed new and virulent populations which overcame the resistant hybrids

The different sunflower genotypes (hybrids, lines and populations) have been tested for resistance to broomrape attack, with a view to identify new sources of resistance to the most virulent populations of this parasite and to establish a new differential set for the parasite races.

The testing has been performed in natural infested areas with this parasite as well as under artificial inoculation using broomrape seeds proceeded from three areas of infection in Romania.

There are three important areas infested with broomrape *(Orobanche cumana*) in Romania, different as presence of different populations of the parasite and infestation degree.

The behaviour of some commercial hybrids, in the natural infestation conditions, in 2009 year, has showed an increasing of the virulence of the parasite in Constanta area. The parasite is more virulent in Tulcea and in Constanta areas, comparing with Braila-Ialomita. Some hybrids resistant to the race F, have been high infested (89.1 and 47.9%) in Tulcea and in Constanta area. In Braila-Ialomita area, the infestation degree was not so high, some hybrids being full resistant.

The testing of resistance under artificial inoculation conditions, with different broomrape sources from Romania has emphasized the different behaviour of some sunflower genotypes (lines, populations and hybrids) to the attack of different broomrape sources.

Key words: broomrape – races – sunflower – virulence

Screening species of the *Helianthus* genus for resistance to broomrape

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ABSTRACT

Broomrape has become one of the most important threats to the cultivated sunflower. New sources of resistance genes are needed to maintain sunflower production. The objective of this research was to evaluate accessions of wild sunflower species and their F_1 interspecific hybrids with cultivated sunflower for resistance to race E.

Plant material consisted of 6 accessions of perennial species and 14 F_1 accessions between perennial species and cultivated sunflower, as well as 42 accessions of annual species. Cultivated line JM8 was used as a sensitive check. Accessions were screened in the greenhouse in the season of 2008/09 and in a field trial during 2009. Plants were grown in the infested soil and evaluated for reaction at the end of the vegetation.

The cultivated check was susceptible in the greenhouse and in the field trial. The annual species showed varying resistance with *H. annuus* and *H. argophyllus* as the most sensitive with an average of 6.6 and 8.5 broomrape plants per sunflower plant. Only 2 out of 7 *H. petiolaris* accessions were susceptible. *H. neglectus* performed well in the greenhouse with only one broomrape plant infecting one plant of the four tested accessions, but accession NEG1363 was infected in the field trial leaving NEG457 and 1183 as resistant. All accessions of perennial species except for an F_1 hybrid of DEC B and F_1 RIG 707 showed complete resistance.

New potential sources of broomrape resistance genes have been found among wild species and their interspecific hybrids.

Key words: broomrape – resistance – sunflower – wild species.

INTRODUCTION

Cultivated sunflower was obtained through a selection process where seed yield and oil content were mostly emphasized (Škorić, 1988). Its quality as a cultivated plant was greatly improved in a relatively short period between 1930 and 1970 where it started from tenth and reached second place as an oil cultivar right behind soybean. Oil content was raised from 28% (1920) to almost 50% (1955), but the disease problem was always present (Heiser, 1976). Broomrape has now become one of the most important threats to the sunflower cultivation. At present, this parasitic angiosperm can be found in Eastern Europe, Spain, the Middle East and India. The yield loss depends on the severity of infection and it can be very high.

Wild species of sunflower represent the source of resistance to many pathogens that attack cultivated sunflower. Because of that, it was reasonable to use wild species as a source of resistance genes in sunflower breeding programs through interspecific crosses. Genetic resistance to broomrape has been introduced to cultivated sunflower from the wild species, mainly *H. tuberosus*, into early cultivars in the former USSR (Pustovoit, 1966). The wide use of the same resistance type led to the appearance of new races (Skoric, 1988). By continuing the broomrape race nomenclature given by Vranceanu et al. (1980) for five physiological races (A, B, C, D and E), a total of seven races with race G as the newest one is described (Molinero-Ruiz and Melero-Vara, 2004), even though the last one could only be a population of race F (Molinero-Ruiz et all. 2009).

According to Acimovic (1977), broomrape was described for the first time in Serbia in 1951, and it started causing significant damage in susceptible hybrids since the 1990s (Masirevic and Medic-Pap, 2009). First report of race E in Serbia was in 1996 (Mihaljcevic). It was determined using a set of differential sunflower lines and broomrape seed collected in the fields in the northern part of Vojvodina. According to recent research, race E is still the most virulent race present in Serbia (Dedic et al. 2009).

Because of the constant increase in selection pressure against broomrape races through introduction of new resistance genes and cultivation of resistant hybrids, new broomrape races are also occurring in shorter intervals and limit the durability of introduced resistance. The strategy of broomrape control is therefore changing, with the aim to make resistance more durable by including the use of herbicide

tolerant IMI cultivars, quantitative/horizontal resistance, by understanding the genetic structure and variability of *O. cumana* populations (Fernandez-Martinez et al. 2009).

Genetic resistance is still the main component in sunflower broomrape control and resistance genes originating from wild sunflower species can have an important role in providing durable resistance. A high level of resistance to broomrape races was previously reported in wild species like *H. tuberosus*, *H. annuus* (Fernandez-Martinez et al. 2008). The objective of this research is to distinguish accessions that are potential carriers of resistance gene/genes for race E by testing the wild species and their F_1 generations with cultivated sunflower. Although the resistance is more frequently found in the perennial species, the annual species are included in the test because they are much easier to cross with cultivated sunflower.

MATERIALS AND METHODS

Plant material consisted of 42 accessions of annual species (Tab. 1) as well as 6 accessions of perennial species and 14 F_1 accessions between perennial species and cultivated sunflower (Tab. 2). Cultivated line JM8 with no resistance genes was used as a sensitive check in the evaluated material. Accessions were screened in the greenhouse in the season of 2008/09 and in a field trial in 2009 for resistance to broomrape.

To promote germination the seed hull of wild species was removed (Chandler and Jan, 1985). Dehulled seeds were first kept in dark in a growth chamber for 24h and after the root emergence transferred to jiffy 7 pots. Seedlings were planted in jiffy 7 pots and grown in the greenhouse at 21ºC (day and night), relative humidity around 80%, and constant light until the phase of two pairs of leaves, after which they were transferred to buckets (10 dm^3) on October 15th 2008 or planted in the field for the field trial in 2009. The buckets were filled with a mixture of sand:perlit:peat in a 1:1:1 ratio which was homogeneously infested with broomrape seeds of race E at a rate of 70 mg/dm^3 soil mixture. Broomrape seeds were extracted from the stalks collected in August 2007 from locations in Vojvodina where race E was previously determined. Plants were grown in the infested soil for 90 days at a photoperiod of 16/8h day/night with natural light supplemented with metal halide lamps. In the field trial, the seed material was sawn at the same time as the rest of the cultivated sunflower. Reaction of plants was evaluated when they reached physiological maturity.

Plants with emerged or underground broomrape stalks were considered susceptible and those without a broomrape stalk as resistant. Severity was calculated as the number of broomrape plants per sunflower plant. Accessions that were susceptible in the green house test were not included in the field trial.

RESULTS AND DISCUSSION

The cultivated check was susceptible in the green house and in the field test. The annual species showed varying resistance with *H. annuus* and *H. argophyllus* as the most sensitive with an average of 6.6 and 8.5 broomrape plants per sunflower plant, respectively. All accessions of those two species were found susceptible in the greenhouse trial. First broomrape stalks emerged after 70 days in two accessions of *H. annuus* and one of *H. niveus* and the rest emerged until day 93 of growing in the greenhouse. Broomrape emergence could be taken as additional information on broomrape resistance, especially for heterogeneous material like the accessions of wild species (Tab. 1).

Table 1. Genotype, acc. identifiers and reaction of annual species to race E of *Orobanche cumana* Wallr.

Species	IFVCNS population	PI number	Greenhouse		Field trial	
			Intensity of attack	Broomrape emergence (DAS[§])	Intensity of attack	Broomrape emergence (DAS)
H. debilis	***1134***	***PI 468678***	***0***	-	-	-
H. debilis	1136	PI 468680	0	-	2.4	79
H. debilis	1290	PI 468687	8	*	-	-
H. debilis	***1675***	***PI 468692***	***0***	-	-	-
H. debilis	1810	PI 494583	3.5	93	-	-
H. debilis	1848	PI 494589	0	-	3.5	97
H. annuus	1970	PI 531015	1.5	86	-	-

H. annuus	2034	PI 547165	8	74	-	-
H. annuus	2104	PI 586809	11.5	74	-	-
H. annuus	2150	PI 586839	9	70	-	-
H. annuus	2191	PI 586866	0.5	-	-	-
H. annuus	2206	PI 586877	3.5	70	-	-
H. annuus	2220	PI 586883	12	74	-	-
H. argophyllus	1317	PI 468649	4	93	-	-
H. argophyllus	1575	PI 468651	6.5	93	-	-
H. argophyllus	1807	PI 494573	15	86	-	-
H. petiolaris	***338***	***PI 435803***	***0***	-	***0***	-
H. petiolaris	***722***	***PI 435831***	***0***	-	***0***	-
H. petiolaris	815	PI 435845	0	-	2.1	93
H. petiolaris	1383	PI 468811	1.5	80	-	-
H. petiolaris	***1910***	***PI 503232***	***0***	-	***0***	-
H. petiolaris	***2146***	***PI 586918***	***0***	-	***0***	-
H. petiolaris	***2208***	***PI 586931***	***0***	-	***0***	-
H. praecox	1142	PI 468851	0	-	7.1	79
H. praecox	1151	PI 468854	0.5	*	-	-
H. praecox	1801	PI 494606	1.5	*	1.8	79
H. praecox	1333	PI 468865	0	-	5.2	86
H. praecox	***1341***	***PI 468849***	***0***	-	***0***	-
H. praecox	1168	PI 468847	0	-	7	90
H. praecox	1821	PI 494610	0,5	*	0	-
H. niveus	1403	PI 468784	0	-	3.9	86
H. niveus	1452	PI 468788	6,5	70	-	-
H. neglectus	1181	PI 468765	0,5	*	-	-
H. neglectus	1363	PI 468775	0	-	5.6	86
H. neglectus	***457***	***PI 435761***	***0***	-	***0***	-
H. neglectus	***1183***	***PI 468767***	***0***	-	***0***	-
JM 8	Cultivated		2,3	86	7.8	79

* All broomrape stalks were underground at the moment of evaluation
§ DAS – Days after sowing

Similar findings in regard to the resistance of *H. annuus* and *H. argophyllus* were presented by Fernandez-Martinez et al. (2008) who also described species *H. petiolaris* and *H. praecox* as 100% susceptible. *H. petiolaris* performed different in this trial with only 2 out of 7 accessions susceptible. Accession PET1383 had 1.5 broomrape plants per sunflower in the greenhouse and was not tested in the field and accession PET815 was not infected in the greenhouse but it had 2.1 broomrape plants per sunflower of infected plants in the field trial. Different resistance could be simply a result of different accessions that were used or different broomrape race composition.

H. debilis had two resistant accessions DEB1134 and DEB 1675 out of six tested. The rest were infected either in the greenhouse (DEB1290, 1810) or in the field trial (DEB1136, 1848). *H. neglectus* performed very good in the greenhouse, with only one broomrape plant infecting one plant of the four tested accessions, but accession NEG1363 was infected in the field trial leaving NEG457 and 1183 as resistant.

All evaluated accessions of perennial species showed complete resistance. Similar results for perennial species was obtained by other authors (Fernandez-Martinez et al. 2000) which is why they were not included in the field trial to limit the possibility of unwanted propagation. It is worth noting that only two out of 14 tested F_1 crosses between cultivated sunflower and perennial species was susceptible. A F_1 hybrid with DEC B accession reached physiological maturity in the greenhouse after approximately 90 days with an attack intensity of only one underground broomrape stalk per sunflower. A F_1 hybrid with RIG 707 was not infected with broomrape in the greenhouse but in the field trial it had 2.1 broomrape plants per sunflower (Tab. 2).

Table 2. Genotype, accession identifiers and reaction of perennial species and their F_1 hybrids with cultivated sunflower to race E of *Orobanche cumana* Wallr.

Genotype / wild species	IFVCNS population	(Wild parent) PI number	Greenhouse		Field trial	
			Intensity of attack	Broomrape emergence (DAS§)	Intensity of attack	Broomrape emergence (DAS)
F1 *H. rigidus*	1693	-	0	-	0	-
F1 *H. rigidus*	1696	-	0	-	0	-
F1 *H. rigidus*	707	-	0	-	2.1	79
F1 *H. tuberosus*	1698	-	0	-	0	-
F1 *H. tuberosus*	1701	-	0	-	0	-
F1 *H. tuberosus*	6	-	0	-	0	-
F1 *H. strumosus*	1927	PI 503249	0	-	0	-
F1 *H. strumosus*	1623	PI 468894	0	-	0	-
F1 *H. divaricatus*	2085	PI 547174	0	-	0	-
F1 *H. hirsutus*	1536	PI 468738	0	-	0	-
F1 *H. eggertii*	1626	PI 468712	0	-	0	-
F1 *H. decapetalus*	B	-	0.5	*	0	-
F1 *H. resinosus*	1545	PI 468879	0	-	0	-
F1 *H. laevigatus*	1618	PI 468740	0	-	0	-
H. tuberosus	675	PI 468829	0	-	-	-
H. tuberosus	26	-	0	-	-	-
H. grossesseratus	56	-	0	-	-	-
H. mollis	1530	PI 468760	0	-	-	-
H. nuttalii	239	PI 435779	0	-	-	-
H. tuberosus	CG 56	-	0	-	-	-
JM 8	Cultivated		2,3	86	7.8	79

* All broomrape stalks were underground at the moment of evaluation

§ DAS – Days after sowing

It has been shown that the wild species and especially perennials have a great potential in providing resistance for the future broomrape races. Although the resistance was higher in perennial species and their F_1 progenies, resistant plants found among the annual species are very important in regard to the short-term breeding programs. It is always better to use the resistance genes from the gene pool of cultivated sunflower but in some case like with the newest races of broomrape, it is fortunate that the interspecific crosses are possible inside the *Helianthus* genus.

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III. NEW METHODS OF DEVELOPING PARENTAL MATERIAL FOR SUNFLOWER BREEDING ON RESISTANCE TO DISEASES

Results of the evaluation of sunflower`s samples from the VIR collection on resistance to races of the Downy mildew agent widespread in Krasnodar region of Russian Federation

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ABSTRACT

Downy mildew caused by the obligate fungal parasite *Plasmopara halstedii* (Farl.) Berl. et de Toni is one of the most harmful sunflower diseases. Loss of young plant caused by pathogen may be up to 80 %. Researches held in Krasnodar region of Russian Federation by Antonova et al. (2006, 2008) identified 7 races of pathogen and showed that against the background of the dominant race 330 races 710 and 730 are also economically significant.

Resistance of 420 sunflower samples taken from the VIR collection on indicated races was analyzed. The majority of them were susceptible to these three races. However, there were samples found which are resistant to separate races as well as to all three ones in complex. Eleven samples showed resistance only to race 330, three – to race 710 and nineteen – to races 330 and 730 simultaneously.

Of greatest value are samples resistant simultaneously to all three races of pathogen. There are 21 detected: local samples VIR 160, VIR 172, VIR 247, VIR 381, VIR 387, VIR 206, VIR 581, VIR 632, VIR 635, VIR 4354, VIR 463 L., as well as HA232 x H.gigantheus and № 2315; foreign samples: Ukrainian, Canadian, Spanish, French, Australian (respective VIR catalogue numbers – 2771, 2306, 2644, 3009, 3362, 3246) and three American ones: № 3532, lines HA-89 and Rha 278.

Thus, in the collection of VIR there were determined valuable samples of sunflower with complex resistance to races 330, 710 and 730 as well as to each race taken separately.

Key words: sunflower – samples from the VIR collection – resistance – susceptibility – downy mildew – races – *Plasmopara halstedii*.

INTRODUCTION

Downy mildew caused by the obligate fungal parasite *Plasmopara halstedii* (Farl.) Berl. et de Toni is one of the most harmful sunflower diseases. Yield loss under conditions favorable for the pathogen development constitutes 50–70 %. From the result of the conducted monitoring in Krasnodar region for many years, the loss of plants as a result of pathogenic infection constitutes up to 80 %.

According to the recent figures, there are 36 pathotypes in the world population of *P. halstedii* (Gulya, 2007). Among them we can single out about 6–7 world dominant races.

Seven races of pathogen were identified during the research conducted by Antonova et. al in Krasnodar region of Russian Federation (2006, 2008), and it was determined that against a background of dominant race 330 races, 710 and 730 are also economically significant. A conclusion was made about the necessity of separate testing on resistance of sunflower to these races and extraction of a material with complex resistance to them. The aim of the present experiment was to analyze the resistance of sunflower samples from the VIR collection to three mentioned races.

MATERIALS AND METHODS

Achenes of sunflower samples from the VIR collection wrapped in rolls of absorbent paper till the size of rootlet 1–2 cm were kept at 25 ^{o} for germination. Seedlings, 25 units of each sample, were placed in rows in plastic germinating cabins filled with river sand covered with absorbent paper. The suspension of zoospores of races 330, 710 and 730 (each race separately) in concentration 10^6 per 1 ml was prepared

through washout of zoosporangiums from the refrigerated till -80 °C cotyledon leaves with sporulation of *P. halstedii*.

Cotyledon leaves with sporulation were reisolates of fungus with known racial belonging. Racial belonging of the used isolates was determined according to famous methodology offered in the 15th International Sunflower Conference (Tourvieille de Labrouhe, et al., 2000). Suspension was pour into the germinating cabins at the rate of 150 ml per one (There was a separate germinating cabin for each race). Plants were grown at 25 °C in the daytime and 18 °C at night for 7-9 days, then they were poured heavily; air temperature was reduced to 16 °C and a moist chamber was made by covering germinating cabins with polyethylene film at the night. Presence of sporulation on the first couple of true leaves, cotyledons or stems was taken into account.

RESULTS AND DISCUSSION

In many decades, the world collection of VIR has been the source of the initial material for breeding. In case of need, breeders can find resources of required genes in the collection, including those which are relevant to new high-virulent races of pathogens.

421 samples from the VIR collection were evaluated on resistance to races 330, 710 and 730 of *P. halstedii*. Among them, 124 samples are developed in 16 far-abroad (France, Canada, USA, Romania, Spain, Morocco and others) and two near-abroad (Ukraine, Kazakhstan) countries. Local samples are developed in different regions of Russia, mainly in Krasnodar region.

The majority of examined samples were determined as susceptible to all three races. Nevertheless, there were samples in collection which revealed themselves as resistant to races taken separately as well as to all three ones in complex. Altogether resistance was shown by 54 samples. Some of them are presented in Table 1.

Among resistant forms - local ones, in Krasnodar region, in nearby country – Ukraine, in far away countries – France, USA and in Canada, Spain, Australia per one sample. Eleven samples showed resistance only to race 330, three – to race 710, and nineteen – to races 330 and 730 simultaneously.

Of greatest value are samples resistant simultaneously to all three races of pathogen (Table 1). There are 21 detected: local samples VIR 160, VIR 172, VIR 247, VIR 381, VIR 387, VIR 206, VIR 581, VIR 632, VIR 635, VIR 4354, VIR 463 L., HA232 x H.gigantheus and № 2315; foreign samples: Ukrainian, Canadian, Spanish, French, Australian (respective VIR catalogue numbers – 2771, 2306, 2644, 3009, 3362, 3246) and three American ones: № 3532, lines HA-89 and Rha 278.

Table 1. Samples of sunflower from the VIR collection which showed resistance to races of *P. halstedii*.

№	Sample			Affection of sample by race, %		
	Number according to the catalogue	Name	Origin	330	710	730
1	358	Gigant 549	Krasnodar region	0	33,3	41,7
2	3220	VIR 160	Krasnodar region	0	0*	0
3	3286	VIR 196	the same	0	100	0
4	3287	VIR 197	- ″ -	0	40	0
5	3314	VIR 247	- ″ -	0	0	0
7	3324	VIR 263	- ″ -	0	50	33,3
8	3333	VIR 378	- ″ -	0	100	100
9	3336	VIR 381	- ″ -	0*	0	0*
10	3338	VIR 387	- ″ -	0	0	0
11	3352	VIR 206	- ″ -	0	0*	0*
12	3381	VIR 581	- ″ -	0	0	0
13	3384	VIR 584	- ″ -	0	100	0
14	3440	VIR 631	- ″ -	0	100	0
15	3441	VIR 636	- ″ -	0	100	0
16	-	VIR 632	- ″ -	0	0	0
17	-	HA 232xH.gigantheus (F10)	- ″ -	0	0*	0
18	-	VIR 436 L	- ″ -	0	0*	0
19	3349	Leader	Krasnodar	33,3	0	50
20	2771	-	Ukraine	0	0	0
21	3339	-	Ukraine	0	71,4	57,1
22	3342	-	the same	0	100	66,6
23	3347	-	- ″ -	0	91,7	75
24	2306	-	Canada	0	0	0
25	2644	-	Spain	0	0	0
26	3009	-	France	0	0*	0*
27	3065	-	France	0	100	100
28	3067	-	France	0	100	100
29	2400	-	USA	0	100	0*
30	3245	-	USA	0	100	0
31	3246	-	the same	0	0*	0
32	3255	-	- ″ -	0	57,1	0
33	3258	-	- ″ -	0	71,4	0
34	3532	HA-89	- ″ -	0	0	0
35	-	RHA-278	- ″ -	0	0	0
36	3362	-	Australia	0	0	0

* - sample is conditionally resistant, because there was slight presence of spores of *P. halstedii* on cotyledons or on stems of some young plants

Characteristics of other breed traits of samples determined as resistant to *P. halstedii* races are presented in table 2.

Resistant lines VIR 196, VIR 263, VIR 378, VIR 381, VIR 387, VIR 581, VIR 584, VIR 631, VIR 636, VIR 632 and Rha 278 are pollen fertility restorers; they are well adjusted and can be used as male parents by hybridization. Interspecific hybrid HA232xH.gigantheus presented by the tenth generation does not split and restores the fertility of pollen CMS RET-1 (ЦМС РЕТ-1). Lines VIR 436 and HA 89 have sterile analogues.

Thus, in the collection of VIR there were singled out valuable samples of sunflower with complex resistance to races 330, 710 and 730 as well as to each race taken separately.

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Table 2. Characteristics of the sunflower samples from the VIR collection which showed resistance to races of *P. halstedii* (according to data received from the Kuban experimental station of VIR, 2008)

№	Number according to the VIR catalogue	Name, origin	Vegetation period, days	Productivity of one plant, g	Mass of 1000 seeds, g	Plants height, см		Diameter of head, cm	Branching, %
						Total	Without head		
	3220	VIR 160	96	-	-	106	104	9	100
	3286	VIR 196	102	-	-	112	88	24	0
	3287	VIR 197	103	-	-	128	115	14	89
	3314	VIR 247	106	-	-	141	122	12	100 lower
	3324	VIR 263	104	57	35	118	81	10	100
	3333	VIR 378	100	-	-	-	-	-	100
	3336	VIR 381	98	-	-	150	120	13	100 lower
	3338	VIR 387	105	71	58	164	135	15	0
	3352	VIR 206	106	-	-	79	67	19	100 lower
	3381	VIR 581	100	42	39	120	106	15	100 upper
	3384	VIR 584	100	-	-	112	104	13	100
	3440	VIR 631	97	35	24	122	115	10	100
	3441	VIR 636	102	40	32	134	126	9	100
	-	VIR 632	100	65	37	113	111	11	100
	3567	HA232xH.gigantheus (F_{10})	95	20	33	101	74	16	100 lower
	-	VIR 436 L	115	47	62	140	133	17	0
	3349	Leader	115	100	72	210	192	28	0
	3532	HA-89 (USA)	104	62	46	109	95	18	0
	-	RHA-278 (USA)	104	72	33	155	137	12	100
	3339	Ukraine	109	30	49	112	102	16	0
	3342	Ukraine	94	45	43	141	109	18	0
	3347	Ukraine	102	46	39	172	155	11	100
	2644	Spain	105	-	-	136	110	13	66
	3065	France	111	-	-	170	160	14	100
	K-3067	France	102	-	-	127	115	12	100
	3245	USA	106	-	-	-	-	-	100
	3246	USA	107	-	-	-	-	-	0
	3255	the same	106	117	82	157	120	23	0
	3258	- ″ -	115	95	63	146	124	22	39
	3362	Australia	105	58	74	142	135	16	0
Standard		variety Master	107	86	69	217	193	25	0

Argentine wild *Helianthus annuus* L. as a genetic resource for Sunflower Chlorotic Mottle Virus (SuCMoV) resistance

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ABSTRACT

In the last sixty years, wild sunflower (*Helianthus annuus* L.) naturalized in the central region of Argentina. The invasive process was accomplished by an increase in genetic variability due to the genetic flow with domestic sunflower and the prairie sunflower (*H. petiolaris* L.). It is possible that the tolerance to abiotic stress and increased disease resistance contributed to the effective colonization of new environments. Sunflower chlorotic mottle virus (SuCMoV), a member of the potyvirus family, is considered an emergent hazard for the sunflower crop under agricultural conditions prevailing in Argentina. Two populations of wild sunflowers collected in La Pampa and Buenos Aires exhibiting increased tolerance (61% and 35%, respectively) were identified after artificial inoculation with SuCMoV. Tolerant individuals from these populations were crossed among themselves and with HA89, A09 and A10 male sterile inbred lines (IL). After two generations of recurrent selection for virus resistance, the same inbred lines were pollinated by individuals classified as susceptible (S) or tolerant (T). The progeny of crosses IL x S showed 98% of susceptibility while IL x T have only 58%. In crosses involving T specimens, 22% of the plants showed attenuated mottled symptoms, considered as a virus resistance mechanism. The selection process has been delayed due to the self incompatibility mechanism characteristic of the wild genotypes. A low proportion of escapes included as immune plants also hindered the selection process. It is expected that these limitations will be overcome by means of crosses with self compatible maintainer inbred lines (B) and adjustments in the inoculation technique.

Key words: artificial inoculation – naturalized sunflower – potyvirus family – sunflower disease.

INTRODUCTION

Virus diseases are not included as broad limitations to sunflower crop (Skoric 1992). In spite of that, Sunflower chlorotic mottle virus (SuCMoV) is an emergent and widely distributed sunflower potyvirus, reported in several provinces of Argentina. Infected plants, found mainly in commercial crops, typically show generalized mottling symptoms on leaf blades and plant stunting (Lenardon et al., 2005b). Infections of SuCMoV occurring at early crop stages significantly reduce the sunflower yield (Lenardon et al., 2001). The taxonomy of this potyvirus, recently analyzed by means of the nucleotide sequence of the genomic 3´ terminal, confirms its previous classification and suggests the existence of several races (Bejerman et al. 2008). A single dominant resistant gene (*Remo-1*), providing a good partial resistance, has been identified in the private inbred line L33 (Lenardon et al. 2005a). Due to the importance of sunflower crop to Argentine economy, public institutions are devoted to develop new and effective strategies to SuCMoV control, including the development of other resistance sources.

After their introduction nearly 1954, wild *H. annuus* dispersed across the central area of Argentina, from S31° 20´ to S37° 31´ latitude (Poverene et al. 2002). The invasive process have been associated with a high phenotypic biodiversity (Cantamutto et al. 2010), probably originated in the intense gene flow with the sunflower crop (Ureta el al 2008) and introgression with other invasive wild species, *H. petiolaris* (Gutierrez et al. 2010). Argentine wild sunflowers could have developed resistance genes under natural selective pressure, due the broad virus diffusion. This work describes the advances and future directions in the detection of SuCMoV resistance sources from wild Argentine sunflowers and its introgression into domestic sunflower pure lines.

MATERIALS AND METHODS

Nine wild *H. annuus* accessions collected during 2002-2003 summer seasons on representative geographical habitats (Cantamutto et al. 2008), expressing different phenotypes in a common garden study (Presotto et al. 2009), were selected to initiate the evaluation and selection procedure.

Evaluation for virus tolerance was performed on plants grown under glasshouse conditions and artificially inoculated at four to six expanded leaves stage during three years. A SuCMoV isolate was maintained on susceptible sunflower plants in the greenhouse and used as the inoculum source. Infected leaves were ground in 0,01M Na_2HPO_4/NaH_2PO_4, pH 7 containing 0,1% Na_2SO_3, and silicon carbide 600 mesh added as abrasive (0.25 g/10 mL slurry). Inoculum was applied with a high-pressure airbrush apparatus previously described (Lenardon et al. 2005a). Two weeks after inoculation, plants showing disease symptoms were classified as susceptible. Incidence values were calculated as percentage of infected plants of total inoculated plants from each population. The commercial hybrid Contiflor 7 was used as susceptible control. Data were analyzed as ANOVA under a randomized complete block design with three replicates (years). Statistical differences among means were determined by Tukey test ($p<0.05$).

At the end of the three year initial evaluation under glasshouse conditions (n = 870), we started the selection process. Inoculated plants from the resistant populations showing no disease symptoms were transplanted to the experimental field. Some individuals developed mottling after transplanting and were discharged. Individuals showing no virus symptoms at reproductive stages were selfed, inter-mated or crossed with the male sterile inbred lines (IL) A10, A09 and HA89, susceptible to SuCMoV (D. Alvarez, *personal communication*). To exclude undesirable pollen, the immature heads were covered with bags before flowering and maintained in this situation up to harvest. Crosses involved the wild resource as maternal parent were made without emasculation because of its characteristic high self-incompatibility.

After two successive generations of crosses and selection, the progeny of each genotype was classified according to the inoculation test reaction. Immune (I) plants showed no visible virus symptoms; tolerant plants (T) expressed several grades of leaf chlorotic mottling (Fig. 1a, b); and susceptible plants (S) showed systemic symptoms of SuCMoV (Fig. 1c). A caveat of this classification consisted in the difficulty to distinguish between immune plants and escapes from the inoculation procedure. Classified individuals were used to pollinate the three male sterile IL. Their progenies were evaluated for virus tolerance under the same inoculation procedure. In this evaluation, tolerant individuals were classified if they showed a clear chlorotic pinpoint, similar to this pure line (Fig. 1a). In case of showed extended chlorotic mottling the tolerant plants were classified as intermediate (T - INT, Fig. 1.b). Data were expressed as a percent of each reaction observed in the progeny of each male parent, analyzed by ANOVA under a complete randomized design and means were compared by Tukey test ($p<0.05$).

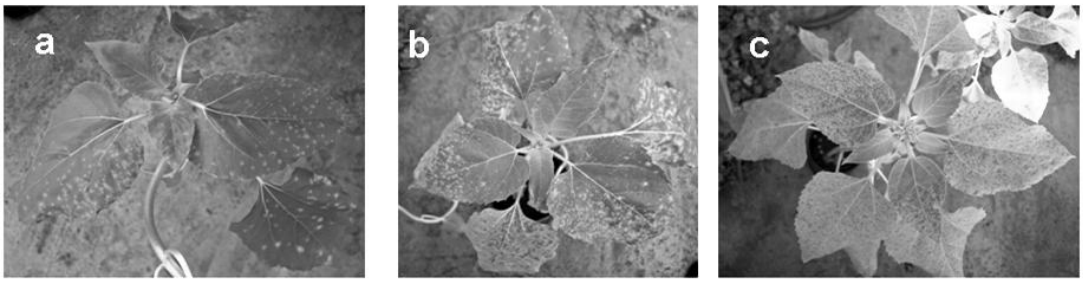

Fig. 1. Wild Argentine sunflowers showing low (a) or intermediate (b) leaf chlorotic mottling and systemic symptoms (c) reactions to SuCMoV artificial inoculation.

RESULTS

Eight out of nine Argentine wild *H. annuus* accessions showed a higher SuCMoV resistance level, different than the control (Table 1). A population from La Pampa province showed the highest resistance level to SuCMoV but without differences from other six wild accessions.

In the first round of crosses, only a few individuals selected from BAR and CHU accessions reached the reproductive stage after transplanted. These individuals did not show any viruses symptoms, and were selfed, sib mated, crossed between them and with the IL. In this round selfing was ineffective to produce viable seed, and only three sibblings were obtained. Crosses between healthy individuals of both resistant accessions produced abundant seed, as did the crosses with the IL. At the second round of crosses, five out of seven sib crosses generate less than five descendants, and selfing was again ineffective to produce seeds. In spite to the low plant number, more than 25% progeny of sib crosses showed virus resistance.

Only thirteen crosses between IL and classified individuals were obtanied. The progeny of crosses between IL and two T genotypes showed a significant higher level of tolerant individuals, expressing L33-like symptoms (Table 2). Eight crosses involving three immune individuals as male parent were excluded from this analysis because they produced inconsistent results, with high levels of susceptibility in their progeny (data not showed).

Table 1: Incidence (%) to SuCMoV artificial inoculation of wild *H. annuus* accessions collected in six Argentine provinces, observed on a three year experiment under glasshouse conditions.

Accession	Code	Province	Incidence	([a])
BAR	4802	La Pampa	39	d
RCU	1403	Córdoba	44	cd
DIA	2303+7002	Entre Ríos	51	bcd
CHU	6402	Buenos Aires	65	bcd
RAN	1802	La Pampa	65	bcd
JCE	4102	Córdoba	65	bcd
LMA	2402	Mendoza	70	bcd
MAG	2702	San Juan	81	bc
AAL	6202	Buenos Aires	87	ab
Check	Contiflor 7	----------	100	a

([a]) Means followed from the same letter are not different according to Tukey test ($p<0.05$).

Table 2: Reaction of five crosses between inbred lines (IL) and four selected genotypes with SuCMoV tolerance (T) or susceptibility (S). Progeny was classified as T - L33 if showed small chlorotic pinpoint (Fig. 1.a), T - INT if showed systemic chlorotic mottling (Fig. 1.b), S if showed generalized chlorosis (Fig. 1c) and I if no symptoms were showed. Into each column, means followed by the same letter are not different at $p = 0.05$ (Tukey).

Cross	Progeny reaction (%)			
	T - L33	T - INT	S	I
IL x T (n=3)	22 a	16	58 b	4
IL x S (n=2)	1 b	0	97 a	1

DISCUSSION

The Argentine wild *H. annuus* showed a high level of SuCMoV resistance and could constitute a source of useful genes. The two resistant accessions were collected on habitats where *H. petiolaris* is also present (Cantamutto et al. 2008). This agroecological situation could have made possible the emergence of novel variability by means of interspecific crosses. For our breeding propose, the introgression of resistant genes from wild sunflower into domestic lines has been strongly limited by their self incompatibility. As no seeds were obtained by self pollination, the increase of the homozygosis in the selected materials has been narrowed. Also, only a few crosses of sib mating produced abundant seed. This restriction in individual evaluation limited the statistical analysis and the inferring of strong hypothesis about the genes involved.

The selective process has been also troubled by the possible existence of escapes to SuCMoV artificial inoculation. As a few escapes could have been included into the immune group, the progeny of their crosses could correspond to a mixture of genotypes. May be for this reason tolerant individuals used as male parents produced healthier progenies than immune individuals. It is clear than a tolerant individual, expressing the virus symptom at low level, poses certain resistance level and this trait seems to be heritable.

In the third selection round, the self incompatibility has been broken. At the present cycle, the progeny of genotypes showing high levels of SuCMoV resistance, obtained by selfing and sib mating of tolerant genotypes has been crossed with emasculated plants of the maintainer lines B09 and A09. The aim was to obtain homozygosis to the resistance genes by selfing their progeny. If self incompatibility were broken, the development of novel inbred lines with SuCMoV resistance could be possible.

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A new gene for resistance to downy mildew in sunflower

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ABSTRACT

Several major resistance genes to downy mildew, caused by *Plasmopara halstedii*, have been either identified in cultivated or wild *Helianthus annuus* or other wild *Helianthus* species. Although these dominant genes confer functionally complete resistance to one or more races of *P. halstedii*, new races continue to emerge. This threat, combined with the development of resistance to chemical control has spurred a vigorous search for determinants specific to new races and for genes conferring broad resistance specificities. Here we report the characterization of a new gene for downy mildew resistance, for which we proposed the name Pl_{15}. This new resistance gene provides resistance to all known races of the pathogen, maps on linkage group (LG) 8 of the sunflower reference SSR map, and has a characteristic haplotype for the resistance gene candidates (RGCs) of the TIR-NBS-LRR subclass in the Pl_1-Pl_2-Pl_6-Pl_7 gene cluster.

Key words: genomic position – resistance breeding – resistance gene.

INTRODUCTION

Several major resistance genes to downy mildew, caused by *Plasmopara halstedii* (Farl.) Berl. et de Toni, have been either identified in cultivated or wild *H. annuus* L. or other wild *Helianthus* species (Korell *et al*., 1996; Miller, 1992). These dominant resistance genes have been designated as *Pl* genes. Some of them provide resistance to a single race of *P. halstedii*, whereas others impart resistance against two or more races (Miller, 1992). Among these, Pl_1 and Pl_2 confer resistance to race 1 (virulence phenotype 100) and races 1 and 2 (virulence phenotype 300), respectively, and trace to early crosses to a wild sunflower population from Texas (Putt and Sackston, 1957).

During decades, most commercial sunflower hybrids were resistant to race 2 of downy mildew (Gulya, 1998), which was the predominant race in the USA until 1981 (Gulya *et al*., 1991) and in Argentina until 1998. As soon as the resistance provided by the Pl_2 was overcome by the pathogen, new resistant genes, mainly derived from public USDA lines were deployed in sunflower cultivars. The Pl_5 gene from *Helianthus tuberosus*, a perennial hexaploid sunflower, protects against race 3 (virulence phenotype 700[1]) (Leclercq *et al*., 1970; Pustovoit, 1966). Resistance to races 1, 2, 3 and 4 (virulence phenotype 730) was introgressed into cultivated sunflower from three sunflower species, *H. annuus ssp. annuus* (Pl_6), *Helianthus praecox* ssp. *runyonii* (Pl_7) and *Helianthus argophyllus* (Pl_8) (Miller and Gulya, 1988, 1991). Also from *H. argophyllus* was introgressed Pl_{Arg}, a gene which conferred resistance to at least four tested races (virulence phenotypes 300, 700, 730, 770). Recently, a new source of resistance derived from HAR5 conferring resistance to nine races of the pathogen has been determined and the new source has been designated as Pl_{13} (Mulpuri *et al*, 2009).

The efectiveness of the major resistance genes Pl_6 and Pl_7 has been overcome by new races in France (Delmotte *et al*. 2008) and in USA (Gulya et al., 2010); however, there are no records of races overcoming other broad spectrum (Pl_8, and Pl_{Arg}) genes (Gulya, 2007; Gulya et al., 2010).

Although these dominant genes confer functionally complete resistance to one or more races of *P. halstedii*, new races continue to emerge. This threat, combined with the development of resistance to chemical control (Gulya *et al*., 1999), has spurred a vigorous search for determinants specific to new races and for genes conferring broad resistance specificities. Moreover, it is not advisable to use only one resistance gene in developing new cultivars. Rather, several different resistance genes should be employed, either by growing different hybrids carrying the different resistance genes or by pyramiding such genes. This strategy may extend the life cycle of each gene by keeping the selection pressure on the pathogen population as low as possible. Strong resistance genes effective against all known races could be overcome soon by new pathogen races if used alone. On the other hand, hybrids that combine strong

[1] Nomenclature of the races according to Tourvieille *et al.*, 2000.

genes with already defeated hypostatic genes may be resistant to such new races (Kelly and Miklas, 1998). Hence, the combination of these defeated genes with novel genes, to which the pathogen has not been exposed, will extend the useful life of the defeated genes and will provide more durable resistance (Lawson *et al*., 1998).

Genetic evidence suggests that four downy mildew resistance genes, Pl_1, Pl_2, Pl_6, and Pl_7 are clustered on LG8 of the sunflower SSR reference map[2] (Mouzeyar *et al*., 1995; Roeckel-Drevet *et al*., 1996; Vear *et al*., 1997). Pl_6 was found to consist of at least two linked (0.6 cM) genetic factors (Vear *et al*., 1997). A second linkage group, LG13, was found to contain two clustered resistance genes, Pl_5 and Pl_8 (Bert *et al*., 2001). The Pl_{Arg} (Dußle *et al*., 2004), Pl_{13} (Mulpuri *et al*., 2009) and Pl_{14} (Bachlava *et al*., 2009) loci, on the other hand, were localized on linkage group LG1.

The objective of this paper is to report the pattern of resistance, genomic position and molecular characterization of a new gene for downy mildew resistance found in the line RNID, a proprietary restorer inbred line which traces back to an Argentine open pollinated population of sunflower.

MATERIALS AND METHODS

Characterization of resistance in inbred line RNID.

RNID was inoculated by the whole seedling immersion method (Miller and Gulya, 1991) with the following races of *P. halstedii*: 300, 330, 710, 730, 770 by Amelia Bertero de Romano; 304, 714, 733, 734, 770 by Tom Gulya (USDA Fargo, USA); 304, 334, 710 by Jean-Luc Madeuf, (Soltis, France), 100, 330, 700, 710, and 730 by the ***National Institute*** for ***Agricultural*** Quality Control (***Tordas, Hungary***).

Inheritance of resistance to downy mildew in RNID

An F2 population from the cross R720 CL-PLus x RNID consisting in 98 individuals was obtained by selfing a single F1 head. RNID is a restorer inbred line which shows resistance to downy mildew and R720 CL-Plus is a downy mildew susceptible, imidazolinone resistant line (Sala *et al*., 2008). DNA was extracted from each individual F2 plant by the method of Dellaporta (Dellaporta, 1983). Each plant was selfed and 98 $F_{2:3}$ families were obtained.

Resistance tests

Tests of $F_{2:3}$ families, RNID and 12 lines derived from it were performed in a growth chamber at 16–21 ◦C and a 13 h photoperiod with 100 μE.m−2.s−1 light. Seeds were surface sterilized, incubated in a germinator and inoculated by the whole seedling immersion method (Miller and Gulya, 1991).

SSR analysis.

PCR reactions were performed in 20 µL of reaction mixture containing 2 µL 10x PCR buffer, 2.5 m*M* Mg^{2+}, 0.2 m*M* each of dNTPs, 7.5 pmol of each primer, 0.7 units of Platinum Taq polymerase (Invitrogene Life Technologies, Carlsbad, CA, USA), and 20 ng of genomic DNA. The microsatellite procedure was described in Tang *et al*. (2002). PCR reactions were then performed in a PTC200 thermocycler (MJ Research, Waltham, MA, USA). The PCR products were separated by electrophoresis in agarose gels [1.5% (w/v) in TBE pH 8.3] or denaturing polyacrylamide gels [6% (w/v) acrylamide/bisacrylamide, 20:1, 8 *M* urea in TBE, pH 8.3]. Gels were stained with a SYBR Gold nucleic acid gel stain (Molecular Probes, Eugene, OR, USA) and visualized in a Fluor-S multimager (Biorad, Hercules, CA, USA).

One hundred eighty microsatellites markers were screened comparing the parental lines and progeny individuals to find polymorphic markers. The markers were scored in 98 F2 individuals from the cross RNID x R720 CL-Plus. Distances between the resistance gene and the molecular markers, were determined by maximum likelihood estimations with the computer program MAPMAKER 3.0 (Lander *et al*., 1987), using default parameters of LOD=3 and a maximum Kosambi distance of 50 cM and the default algorithm.

IFLP fingerprinting

To characterize the resistant gene in RNID at the molecular level we used multilocus intron fragment length polymorphism (IFLP) fingerprinting using a single pair of primers flanking a hypervariable intron located between the TIR and NBS domains (Slabaugh *et al*., 2003)

The HaRGC1 IFLP marker (F97-R98 primer combination) was used to fingerprint three lines carrying already known *Pl* genes, RNID, and twelve downy mildew resistant lines derived from crosses between RNID and susceptible restorer lines (Table 2). Fluorescently labelled PCR products were amplified from the germplasm accessions and separated on polyacrylamide gels using the Licor

[2] References to the Cartisol or other genomic maps of sunflower are referred to the SSR consensus map published by Tang *et al.*, 2002. and Yu et al., 2002.

genotyping platform. Haplotypes were constructed by scoring for the presence or absence of each of the IFLP amplified fragments.

RESULTS AND DISCUSSION

Pattern of resistance

Line RNID was inoculated with 13 different *P. halstedii* races together with an internationally standardized set of nine sunflower differential lines (Table 1). It shows resistant not only to the four predominant races of downy mildew in all sunflower producing countries (300, 700, 730, and 770, Gulya 2007) but also to the less prevalent races, including the races described recently in USA (714 and 734) and in France (304) which overcome Pl_6 (Gulya et al., 2010; Vear, 2004).

Inheritance of resistance to Downy Mildew in RNID

The $F_{2:3}$ families from the cross RNID x R720 were inoculated with *P. halstedii* race 770. Of the 98 $F_{2:3}$ families, 31 were homozygous resistant, 41 were heterozygous and 26 were homozygous susceptible. This segregation ratio of resistance to susceptible fit the Mendelian segregation ratio of 1:2:1 ($\chi^2 = 3.122$, $P = 0.21$) and suggested that downy mildew resistance in RNID is controlled by a single dominant gene or a cluster of several tightly linked genes.

Table 1. Reaction (S, susceptible; R, resistant) of RNID and a set of internationally standardized nine sunflower differential lines to different physiological races of *Plasmopara halstedii*.

	Races												
Lines	**100**	**300**	**304**	**330**	**334**	**700**	**703**	**710**	**714**	**730**	**733**	**734**	**770**
HA 304	S	S	S	S	S	S	S	S	S	S	S	S	S
RHA 265	R	S	S	S	S	S	S	S	S	S	S	S	S
RHA 274	R	R	R	R	R	S	S	S	S	S	S	S	S
DM 2	R	R	R	S	S	R	R	S	S	S	S	S	S
PM 17	R	R	R	S	S	R	R	R	R	S	S	S	S
803-1	R	R	R	R	R	R	R	R	R	R	R	R	S
HAR 4	R	R	R	R	R	R	S	R	R	R	S	R	R
HAR 5	R	R	R	R	R	R	S	R	R	R	S	R	R
HA 335	R	R	S	R	S	R	R	R	S	R	R	S	R
RNID	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**

Cosegregation of resistance to multiple races

Twelve F6 lines derived from crosses of RNID with different susceptible restorer lines and selected during inbreeding with race 770 alone, were inoculated additionally with races 300, 700 and 730, together with lines HA304, RHA274 and HA335 used as checks. The twelve lines showed resistance to these races, which suggest that the gene (or cluster of resistant genes) in RNID, which provides resistance to race 770, also confers resistance to the other races.

Genomic position of the resistant gene in RNID

Of the 180 SSR primer pairs tested, forty-two markers distributed on the 17 linkage groups of sunflower genome were polymorphic between the parental lines RNID and R720 CL-Plus. Assessment of the 42 polymorphic primers on resistant and susceptible bulks and F2 population resulted in the identification of only one SSR marker (ORS 166, in LG8) associated with the downy mildew resistance phenotype. Distance between this marker and the resistant gene in RNID was 3.4 cM. Since ORS 166 was reported to be linked to the Pl_1-Pl_2-Pl_6-Pl_7 gene cluster by Slabaugh *et al*. (2003), the resistant gene reported here is a new member of this cluster.

IFLP fingerprint

Molecular characterization indicated that the Pl_1-Pl_2-Pl_6-Pl_7 region on LG8 of sunflower is a complex locus populated with RGCs of the TIR-NBS-LRR class and multiple disease resistance determinants (Gentzbittel *et al*., 1998; Gedil *et al*., 2001). Primers flanking an intron between the TIR- and NBS encoding regions of an RGC of the TIR-NBS-LRR subclass (named HA-4W2 by Gedil *et al*., 2001 and HaRGC1 by Slabaugh *et al*., 2003) amplify a large family of resistance gene candidates. Slabaugh *et al*. (2003) reported that the DNA fingerprints produced by the F97/R98 primer combination were

characteristic of each of the members of the Pl_1-Pl_2-Pl_6-Pl_7 gene cluster. For this reason, we compared the HaRGC1 haplotype obtained from RNID and RNID-derived lines with those obtained from other resistant lines from the same cluster on LG8 (Table 2).

Table 2. Intron fragment length polymorphism (IFLP) fingerprinting of sunflower line RNID and lines carrying the genes Pl_1, Pl_2, and Pl_6.

	Haplotype			
RGC	Pl_{Rnid}	Pl_1	Pl_2	Pl_6
rgc-1	X			
rgc-2	X	X	X	X
rgc-3	X			
rgc-4		X		
rgc-5			X	
rgc-6	X			
rgc-7	X			
rgc-8	X	X		
rgc-9	X			
rgc-10			X	
rgc-11		X		
rgc-12	X	X		X
rgc-13				X
rgc-14			X	
rgc-15				X
rgc-16				X
rgc-17				X
rgc-18			X	

Ref: Pl_2 was represented by RHA274, Pl_1 by HA370, Pl_6 by HA335 and Pl_{RNID} by the inbred line RNID plus twelve RNID-derived lines.

Eighteen HaRGC1 bands ranging in size from 400 to 1200 bp were observed among the 19 utilized lines carrying the genes Pl_1, Pl_2, Pl_6 and Pl_{RNID}, and designated as rgc1 to rgc18 according to their increase in size (Table 2). Each haplotype could be unambiguously discriminated from the rest, since they shared only one common band (rgc2). The line RNID and all its derived lines showed exactly the same haplotype, consisting in 8 bands, which were different for the other haplotypes of the same gene cluster. In fact, five out of these 8 bands were unique for this new haplotype, since they were absent in the lines carrying the Pl_1, Pl_2 and Pl_6 genes (Table 2).

CONCLUSION

The combined results from the pattern of resistance, inheritance, cosegregation, genomic position, and IFLP fingerprint indicated that the gene present in RNID is a dominant gene (or cluster of resistant genes) for downy mildew resistance in sunflower. This new gene, for which we proposed the name Pl_{15}, shows resistance to all known races of the pathogen, maps on LG 8 of the sunflower reference SSR map and has a characteristic haplotype for the RGCs of the TIR-NBS-LRR subclass in the Pl_1-Pl_2-Pl_6-Pl_7 gene cluster.

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Sources of sunflower resistance to Phomopsis in the VIR collection

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ABSTRACT

Sunflower samples of the VIR collection tolerant to affection by Phomopsis are determined. Of great value are the samples which are not affected by Phomopsis at all according to the results of long-term field observations and under conditions of natural affection and artificial inoculation against the severe infectious background: old variety Zelenka (catalogue number 552) and lines VIR 249, VIR 365, VIR 448 and VIR 449 developed at the Kuban experimental station of VIR. Lines VIR 249, VIR 365 and VIR 448 are lines-restorers of the pollen fertility of CMS PET 1 for breeding on heterosis. Line VIR 449 is ornamental, recorded in the State register of selection achievements as variety Solnyshko. Moreover, according to the results of observations in 2006–2008 lines from 11–13 generations of inbreeding, five lines developed on basis of interspecific hybrids of INRA development (France): RIL 273, RIL 440, LR1, RIL 270 и RIL 342 (derived from the hybrid HA 89 x LRI) and one VIR line from the 14th generation of inbreeding derived from a hybrid (line VIR 114 × *H.gigantheus*) were determined as resistant to Phomopsis. As a result of the research a collection of sources of resistance to Phomopsis including 12 samples has been created.

Key words: Phomopsis – resistance – tolerance – collection – sunflower – samples – interspecific hybrids

INTRODUCTION

Phomopsis of sunflower caused by fungus *Phomopsis (Diaporthe) helianthi* Munt.-Cvetk., Michal, Petr. refers to its most harmful diseases. Pathogen is under the inner quarantine in Russia. In years favorable for the pathogen development yield losses can be 50 %. In that case development of resistant hybrids and varieties of sunflower is the most radical way of solving the problem. During a few decades the world collection of VIR has been being the source of the initial material for breeding.

MATERIALS AND METHODS

Sunflower samples of the VIR collection which have been being collected in the world's main sunflower crop areas in 115 years served as the material for the research. Collection was sown for maintenance and study at the Kuban experimental station of VIR in Krasnodar region. Triple-row plots, each with seven slots, in area of 10,5 m^2. Placement of plants was 70x70, two plants for a slot, total quantity of plants in a plot was 42. Evaluation on resistance is made visually by examination of each plant of a plot three times in every ten days during August and September. Line VK-571 of the VNIIMK development (catalogue number 3511) was sown as a control heavily affected by Phomopsis. Released mid-ripening Master variety (No. 3553) was used as a standard variety for the examination of economic traits of samples.

Affection of samples was determined by the percentage correlation between the quantity of affected plants and total quantity of plants on a plot. Sunflower samples characterized with the resistance to Phomopsis under field conditions were evaluated in VNIIMK by using two methods of inoculation: 1. with ascospores by the severe infectious background and 2. with mycelium into the base of petiole. Such double affection allows to distinguish the resistance of leaves and stems. Resistant hybrid Kubansky 930 and susceptible line VK 571 were used as a control (Antonova et al., 2003).

Infectious background was created by placing fragments of 30 cm long sunflower stems of the previous field season affected by Phomopsis and having wintered outdoors between rows of plants being tested. Plants of the evaluated samples were grown on double-row plots (26 plants per row) and inoculated with mycelium into the base of petiole at the stage of budding. Dynamics of stem affection was being observed throughout three calculations in a month a half, starting in two weeks after the moment of affection. The resistance classification was based on the analysis of the affection dynamics

data in accordance with the scale of the affection degrees and resistance classifier developed in VNIIMK (Antonova et al., 2003).

RESULTS

The sunflower collection of VIR exists from the moment of foundation of the institute in 1922. At the moment the collection contains 2300 samples of cultivated sunflower and 495 samples of wild species, 126 of which are perennials. About 900 samples are sown annually for maintenance of the germinating ability, among them there are about 600–700 samples of cultivated sunflower and about 100 samples of annual species. The collection of cultivated sunflower is presented with old and modern samples, breeding lines from all countries where sunflower crop is developed, local populations collected in the course of expeditions in the territory of Russia, former Soviet Union and foreign countries. Wild species of sunflower came from the North America, but some of them were received from gene banks and research institutes of European countries. All samples are estimated every year on the resistance to all diseases occurring during each field season.

For the first time Phomopsis was detected on the test sowings of the Kuban experimental station of VIR in 1997. Later on this disease has been appearing regularly in every 1-2 years depending on weather conditions. According to our observations, severe lesion of the samples from the collection was observed in 1997, 2000, 2004, 2006, 2009. In these years practically all samples of cultivated sunflower of different geographical and genealogical origin sown for keeping and research were affected to a greater or lesser degree. In many cases it was not possible to get seed yield, or a very low rate of germinating ability was shown. Against this background there were identified eight samples from the collection of cultivated sunflower which are resistant to Phomopsis. There were some resistant samples from the wild species nursery which refer to the following species: *H. divaricatus* L., *H. hirsutus* Raf., *H. grosseserratus* Martens, *H. mollis* Lam., *H .salicifolius* A. Dietr., *H. rigidus* (Cass.) Desf., *H. tuberosus* L.(Gavrilova, Anisimova, 2003).

Necessary condition for the Phomopsis development is a warm and sufficiently damp vegetation period. For example, such was the summer in 2006. Temperature conditions in July were close to standard ones, average monthly temperature was 23.6°C, what is by 0.5°C higher than the average one of many years. Rainfall amount was 61 mm (+5 mm to standard). Warm weather was holding on in August too, average monthly temperature was higher than the average multiannual one by 1.3°C. Rainfall amount was significant: 89 mm at the standard rate of 49 mm. Two first decades of September were warm, but dry. A rapid fall in temperature was in the third decade (13,5°C). Rainfall amount of that decade was 26 mm. In year 2008 428 sunflower collection samples of different geographical origin were sown for the maintenance of germinating ability and examination of the affection by Phomopsis under field conditions. The beginning of Phomopsis lesion of plants was being observed in the first decade of August, and a peak of disease – at the end of this month and at the beginning of September. The overwhelming majority of the examined samples showed high degree of the affection by Phomopsis (table 1). More than a half of them (57%) were affected by 100% and a significant quantity (19.6%) – by 61-80%. The minimum quantity of samples (5) showed the distribution of disease within the limits 11–20%.

The susceptibility of different samples didn't depend on their geographical origin. The majority of them (174) were received from the European countries, just a bit lesser quantity (138) was collected in the territory of the Russian Federation. Distribution of the rest of samples is the following: 46 – from the states of the American continent, 26 – from the Transcaucasia, 12 – from the African countries, seven from China, five from Australia and four from the Iraq (tables 2, 3). All groups which had been received from nine different geographical points had approximately equal percentage of heavily affected samples (for 100%): about 50% or a bit more (Russia, America), in other words, close to the average rate (57%). Exception is demonstrated by samples from Australia (20%) and Iraq (75%). Apparently, this can be explained by the fact that not very much samples from these countries were being used in the course of the research.

Table 1. Degree of affection of the world sunflower collection samples by Phomopsis (Kuban experimental station of VIR, 2008)

Affection, %	Quantity of samples	
	Affected	% of total quantity
0-10	7	1,6
11-20	5	1,2
21-40	17	4,0
41-60	33	7,7
61-80	84	19,6
81-99	38	8,9
100	244	57
Total	**428**	**100**

Table 2. Total affection of samples of different geographical origin (Kuban experimental station of VIR, 2008)

Geographical origin	Quantity of samples		
	Total	100% affected	Percent of total quantity
Europe	174	90	52
Russia	138	87	63
America	46	30	65
Transcaucasia	26	12	46
Central Asia	16	9	56
Africa	12	6	50
China	7	4	57
Australia	5	1	20
Iraq	4	3	75
Total	**428**	**242**	**57**

According to the results of estimation held in 2008 samples which had not been affected by Phomopsis in year 1997 – catalogue numbers 552, 3326, 3469, 3487, 3527 – showed the absence of the lesion, but samples 2336 (from Poland), 2539 (Argentina), 2629 (the USA) and 3135 (Altai region) which had been identified as resistant in 1997 showed susceptibility in 2008. Tolerance of twelve more samples was identified in 2008 (table 3). Of great interest are samples which showed no symptoms of the Phomopsis affection at all: three samples from the European countries, two from the Transcaucaia, and one from Canada – self-pollinated line CM-198. Other samples are populations. Concerning the length of the vegetation period two samples (1883 и 2865) are late ripening, others are mid ripening. Six samples which were slightly affected (from 10 to 20%) in our opinion also deserve attention. They are: variety Smena (2052) developed in Russia, line L-337 from Poland and three populations from France (2346), Belarus (1693) and Moldavia (1971).

Some correlation between the susceptibility of samples to Phomopsis and length of their vegetation period is being detected. According to data received in 2009 distribution of Phomopsis in the nursery of middle-early samples with the vegetation period of 92-106 days was 37 %. In nursery of middle-late large seeded samples (vegetation period – 106–117 days) affection by Phomopsis was 58%. The latest confectionery varieties of the Chinese development (vegetation period – 123–130 days) showed 100% affection. Quantity of samples in each ripeness group was 30–50. At the same time many ultra-early ripening samples with period of 75–80 days between shoots and ripening are not affected by Phomopsis because they manage to finish flowering and formation of achenes by the moment of the disease emergence. We can't rate these samples as resistant until they are tested by a severe infectious background.

Table 3. Sunflower collection samples which showed resistance to Phomopsis (Kuban experimental station of VIR, 2008)

No.	Origin	Catalogue No.	Status	Affection by Phomopsis, %	Vegetation period (shoots – ripening), days
1	France	1883	Population	0	117
2	Germany	1957	Population	0	95
3	Armenia	2219	Population	0	100
4	Ukraine	2678	Population	0	102
5	Armenia	2865	Population	0	116
6	Canada	2302	Line SM 198	0	100
7	France	2346	Population	10	102
8	Russia	2052	Variety Smena	14	100
9	Belarus	1693	Population	14	112
10	Poland	2493	Line L-337	14	91
11	Moldavia	1971	Population	17	101
12	Argentina	2547	Population	20	112

According to the results of the ecological test of lines RILs developed in France on base of interspecific hybrids we have sampled 11 lines which had not been affected by Phomopsis in years 2000–2007 and were included into the collection of the resistance sources in 2008. Under the conditions of 2009 tolerance to Phomopsis was shown only by five of them: RIL 273, RIL 440, LR1, RIL 270 and RIL 342. Other six lines were affected from 3 to 22%. According to the long-term annual observations only 12 samples haven't been affected by Phomopsis (table 4).

Table 4. Sources of sunflower resistance to Phomopsis (Kuban experimental station of VIR, 2009)

Name, origin	Catalogue number	Vegetation period, days	Thousand-seed weight, g	Plants height, cm	Affection by Phomopsis, %
Variety Zelenka	552	100	62	180	0
VIR 365	3326	106	59	150	0
VIR 249	3469	107	62	140	0
VIR 449	3527	105	59	133	0
VIR 448	3487	107	39	120	0
VIR 114 x H. giganteus	3570	98	65	115	0
VIR 130	3595	96	62	162	0
LR1, France	3571	103	28	105	0
RIL 440, France	3614	92	40	120	0
RIL 270, France	3615	103	48	143	0
RIL 342, France	3616	101	38	160	0
RIL 273, France	3617	102	30	170	0
Control, VK - 571	3511	88	63	110	98
Standard variety Master	3553	103	79	197	14

Collection of resistant sources includes old variety Zelenka (catalogue number 552), five lines developed at the Kuban station, an interspecific hybrid VIR 114 x *H. giganteus* of the eleventh inbreeding generation and five lines of French development. The interspecific hybrid is a mid ripening, nonbranched, well adjusted over the morphological traits introgressive line of the eleventh inbreeding generation. Line VIR 130 was sown in quantity of eight generations of different plants. Splitting on resistance to Phomopsis was identified: four generations didn't display any affection symptoms, and four ones had a low affection degree: 3, 4, 8 and 15%. In 2009 four generations of line VIR 130 selected on tolerance to Phomopsis didn't show affection symptoms. The control sample (VK-571, catalogue number 3511) was 98% affected in 2009, standard variety Master – 14%. Some samples of the resistance sources collection have other valuable breeding traits. Lines VIR 365, VIR 249 and VIR 449 are donors of genes of pollen fertility restoration for CMS PET 1 and can be used as paternal forms by developing sunflower hybrids resistant to Phomopsis. Line VIR 448 is ornamental and is recorded in the State register of selection achievements as variety Solnyshko. Line VIR 130 has a sterile analog based on CMS PET 1 and some marker morphological traits: low arcuate branching, anthocyanic color of hypocotyl and tubular flowers, stronger leaf venation, orange-yellow false ray flowers, deformed head, white seeds.

Phomopsis resistance in variety Zelenka (No. 552) and lines developed at the station: VIR 365 (No. 3326), VIR 448 (No. 3487) and VIR 449 (No. 3527) was confirmed by the results of evaluation under conditions of artificial inoculation at the immunity laboratory of VNIIMK (table 5).

Table 5. Evaluation of samples from the sunflower collection at the seed ripening stage on the resistance to Phomopsis under the artificial inoculation (VNIIMK, 2006)

Name of sample	Catalogue No.	Percentage of stems affected by Phomopsis under the inoculation	
		With mycelium into the petiole	With ascospores from the artificial infectious background
Variety Zelenka	552	0	0
VIR 449	3527	4 (2)*	0
VIR 249	3469	5 (2)	0
VIR 365	3326	7 (2)	0
VIR 448	3487	14 (2)	0
VK 571, control		100 (4)	100 (4)

* - In brackets there is given degree of stem affection; degree 2 at the seed ripening stage shows that material is resistant

Thus, old variety Zelenka developed at the Veydelevsky experimental station and lines VIR 249, VIR 365, VIR 448 and VIR 449 developed at the Kuban station as lines-restorers of pollen fertility breeding on heterosis refer to the samples resistant to Phomopsis according to data received in the course of field evaluation and results of testing in the xonditions of severe infectious background.

DISCUSSION

It has been shown earlier that plants resistant to Phomopsis are characterized by the thicker collenchyme, presence of vital cell layer in pith and late lignification of pericycle, that's why early ripening forms of sunflower are affected stronger than late ripening ones (Dozet, 1996; Antonova, 1999). Data given above also indicate the correlation between the resistance of samples and length of their vegetation period. Perhaps, it is caused by different weather conditions during the vegetation period, in particular by the correlation between air humidity and temperature (hydrothermal coefficient) that influence spread of the infection. E.G. Dolzhenko came to the similar conclusion in his work (2000). Phenotypes of resistant varieties and lines from the VIR collection do not correspond to the "stay green" mutation, for which a close correlation with the absence of affection by pathogen has been established (Miller & Fick, 1997). Based on our observations, it should be noted that we can't talk about resistance or susceptibility of the species taken as a whole. Most likely, resistance is attributable to various populations or even plants of given species which should be used as sources of resistance.

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IV. METHODS OF IDENTIFICATION AND SELECTION OF SUNFLOWER PLANTS RESISTANT TO DISEASES

Evaluation of sunflower plants resistance to Rhizopus

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ABSTRACT

Results of evaluation of effect made by affection of sunflower head by Rhizopus on economic traits and sowing qualities of seeds are presented. Scale for determination of resistance degree for evaluation and identification of forms resistant to *Rhizopus* fungi has been developed on basis of obtained findings.

Key words: resistance – fungi – evaluation – immunologic scale – acid value

INTRODUCTION

There are 65 fungal species, ten bacteria, two viruses and four parasitic flowering plants registered on sunflower. However, among all this diversity of the phytopatogenic complex significant economic losses can be made just about by ten diseases. Their harmfulness depends on soil and climatic conditions, crop management, short crop rotation, level of the breeding work.

Rhizopus affects is one of the widespread and harmful diseases in regions with warm climate. High rate of air humidity and temperature 30-35 ^{o}C stimulate faster development of this disease. Disease is registered on sunflower in the following countries: Yugoslavia, Bulgaria, Portugal, Spain, Australia, Iran, the USA, Israel, India, Canada [1].

Losses caused by the Rhizopus pathogen depend mainly on the climatic conditions. Strong disease manifestation is observed in dry and warm regions. Quantity of affected sunflower heads fluctuates between 20 and 100%. Yield losses can be 3-50%. Affection of heads by this pathogen reduces seed yield by 46.8%, quantity of filled seeds by 38.2%, oil content of achenes by 6-10%. There is a reduction of germination capacity by 5-19% [4, 9, 11].

According to the long-term observations conducted by VNIIMK in the Krasnodar region there is an accumulation of *Rhizopus* fungi on sunflower fields and intensification of sunflower plants affection by disease. The strongest disease development was recorded in 1994, 1996, 1999, 2002, 2003, 2005-2009. Quantity of affected heads fluctuated between 35 and 60%. In other years occurrence frequency of Rhizopus was 4-18%. According to the results of our researches, by the intensity of the pathogen development there is an increase of seed huskness by 3-5%, reduction of weight of 1000 seeds by 10%, oil content – by 5.4-6%, germination capacity – by 12-30% [1, 5].

Rhizopus Ehrenb. fungi refer to the Mucoraceae family, Zygomycetes class which is widely spread in nature. Disease is caused by three species of *Rhizopus* Ehrenb. fungi: Rh. oryzae Went et Pringle, Rh. stolonifer Ehrenb., Fr. Vuill., Rh. microsporus V. Tiegh. These are unspecialized, wound, thermophilic toxigenic facultative parasites which have strong damaging effect on nutritional reserves of storage organs and seeds [9, 10, 11, 12, 13].

Harmful effect of Rhizopus on sunflower heads can be reduced by using agronomical and chemical methods. One of the most important ways of controlling this pathogen and increasing quality and quantity of yield is breeding of high-yielding and resistant varieties.

Breeding on resistance to Rhizopus is not used on a large scale due to the absence of efficient methods of artificial inoculation and evaluation of results and reliable differentiation of breeding material resistance to disease.

MATERIALS AND METHODS

In our research we used the most in-demand confectionary variety SPK. Oil acid value was determined according to the State Standard 10858-77. Sowing qualities of seeds (germination energy and laboratory germination) were determined in accordance with the State Standard 12038-84. Economic traits were considered according to technology used in the sunflower variety breeding department in VNIIMK.

RESULTS AND DISCUSSION

Resistance of plants is a quantitative trait. It can be estimated by affection degree, type of immunity and yield losses. It seems to be impossible to identify it on plants affected by Rhizopus by using methods of recording spread of disease (quantity of affected plants). Rhizopus on sunflower head does not cause plant's death. It is hard and unreliable to evaluate resistance to Rhizopus fungi by type of immunity, i.e. by plants response to the invasion of pathogen. Visible destruction of head's tissues not always shows the intensity of pathogenical process. Evaluation by yield losses helps only to determine tolerance of sunflower plants to pathogen. Moreover, yield comparison demands protection of control (non-affected) plot, because pathogen spreads rather quickly by air and affects all near-standing plants. It is easier to conduct an evaluation if there is a correlation between plants' resistance and display of any morphological or biochemical trait. Response of sunflower plant to Rhizopus can be visually described by measuring area of head's surface affected by pathogen. However, it is impossible to conduct a precise differentiation of immunological response of plants to affection. Researchers need quantitative indices showing degree of resistance and affection in points and percents. In VIR there were developed unified 9-point scales of plants' resistance evaluation. These scales are based on verbal description of gradations. The highest grade – nine points – corresponds with the absolute resistance, one point is for the maximum susceptibility. Unified 8-point scale is used abroad for evaluation of affection by fungi. Nevertheless, all numerous scales were developed for the obligate parasites and do not suit facultative ones.

For identification of the quantitative trait associated with affection area and resistance of sunflower plants to Rhizopus we have studied economic and biochemical traits of seeds gathered from heads with different affection degrees.

At a ripening stage we selected healthy and affected heads with symptoms of *Rhizopus* development. According to the head's affection area all plants were divided into five groups (the first group – control one, without symptoms of affection by pathogen; the second one – plants with heads affected 25%; the third one – 50%; the fourth one – 75%; the fifth one – heads are totally affected by pathogen). Thrashing of heads was conducted manually in groups. Seeds were analyzed for oil content, weight of 1000 seeds, oil acid value and seeds germination.

According to the results of estimation reduction of seed weight from plant by 19.8 g and weight of 1000 seeds by 16.8 g can be observed by 25% affection of sunflower head's area. By 50% affection there is a reduction of seed oil content by 3.7% and increase in oil acid value more than twice as much in comparison with the control. Degradation of economic traits and oil seed quality is observed by the 100% affection of head's surface and invasion of mycelium into the achene. Oil acid value increased in 10 and more times in comparison with the control (Table 1.)

Table 1. Effect of sunflower heads' affection by Rhizopus on economic traits and sowing qualities of seeds.

Krasnodar, VNIIMK, variety SPK, 2006-2008.

Affected head area,	Weight of seeds from head,	Weight of 1000 seeds,	Oil content of absolutely dry seeds,	Oil acid value,	Sowing qualities of seeds %	
					Germination energy	Laboratory germination
%	g	g	%	mg KOH		
0	195.8±23.2	158.0±9.6	43.0±2.2	0.8±0.2	82.0±3.1	89.0±2.4
1-25	176.0±14/3	141.2±3.8	41.1±0.6	1.2±0.1	76.0±3.3	83.0±4.1
26-50	140.4±12.3	126.6±9.4	39.3±0.9	1.9±0.5	67.0±5.0	74.0±5.5
51-75	120.8±11.1	110.9±8.2	37.1±1.8	2.7±0.6	59.0±5.6	66.0±7.8
76-100	95.5±21.3	91.2±17.2	31.1±5.0	10.2±5.4	41.0±4.2	52.0±8.7

Pathogen had negative effect on sowing qualities of seeds. Depending on affection intensity germination energy reduced by 6-41%, germination capacity – by 6-37%.

In the process of examination a clear correlation between the laboratory germination and disease spread over seeds was established: the more seeds are affected by Rhizopus, the lower is their laboratory germination (r = - 0.916) (Fig. 1). However, it is impossible to use reduction of laboratory germination observed by the affection as an indirect quantitative index, because this trait depends on many other factors and is not inherited.

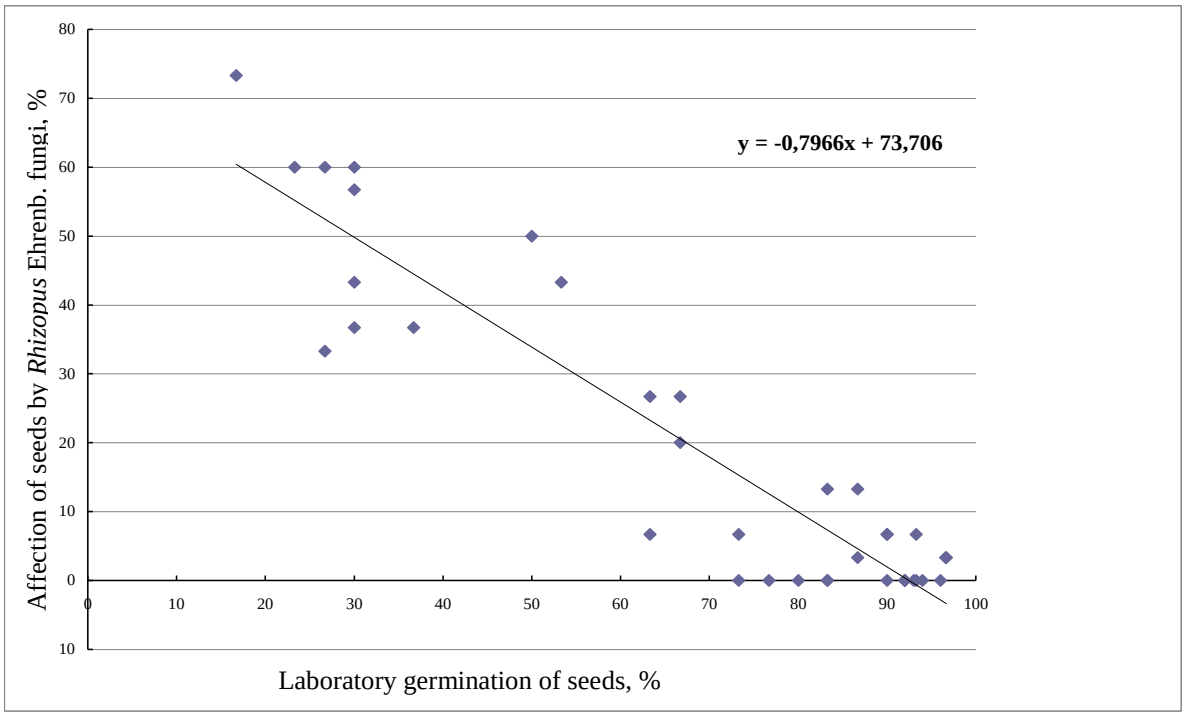

Fig. 1. Dependence of laboratory germination of seeds of the variety SPK on affection by *Rhizopus* Ehrenb.

Another trait closely associated with the intensity of affection by Rhizopus is oil acid value. It is well-known that one of the reasons for the increase of free fatty acids amount in oil is destruction of seeds lipidic complex under the effect of organic acids released by toxigenic fungi. Acid value is a quantitative index of presence of free fatty acids in seed oil. Furthermore, it was established that there is a correlation between the susceptibility of plants to affection and toxins derived by pathogens which remains in each generation [2, 7, 8, 10].

Capability to use oil acid value as an indirect quantitative trait of resistance was checked on heads with affection area of 50% (Fig. 2).

According to the results of examination of seeds taken from the lesion zone oil acid value exceeded 25 mg KOH and laboratory germination were equal to zero. Oil acid value of seeds adjacent to the lesion exceeded 5.5 mg KOH, laboratory germination fluctuated from 10% to 30%. Oil acid value of visually healthy seeds didn't exceed 0.5-1.2 mg KOH, laboratory germination – 83-92%. On average in heads, oil acid value was about 2 mg KOH, laboratory germination – 72%.

Research showed that oil acid value to a considerable degree depends on the intensity of seed affection. These traits are closely interrelated.

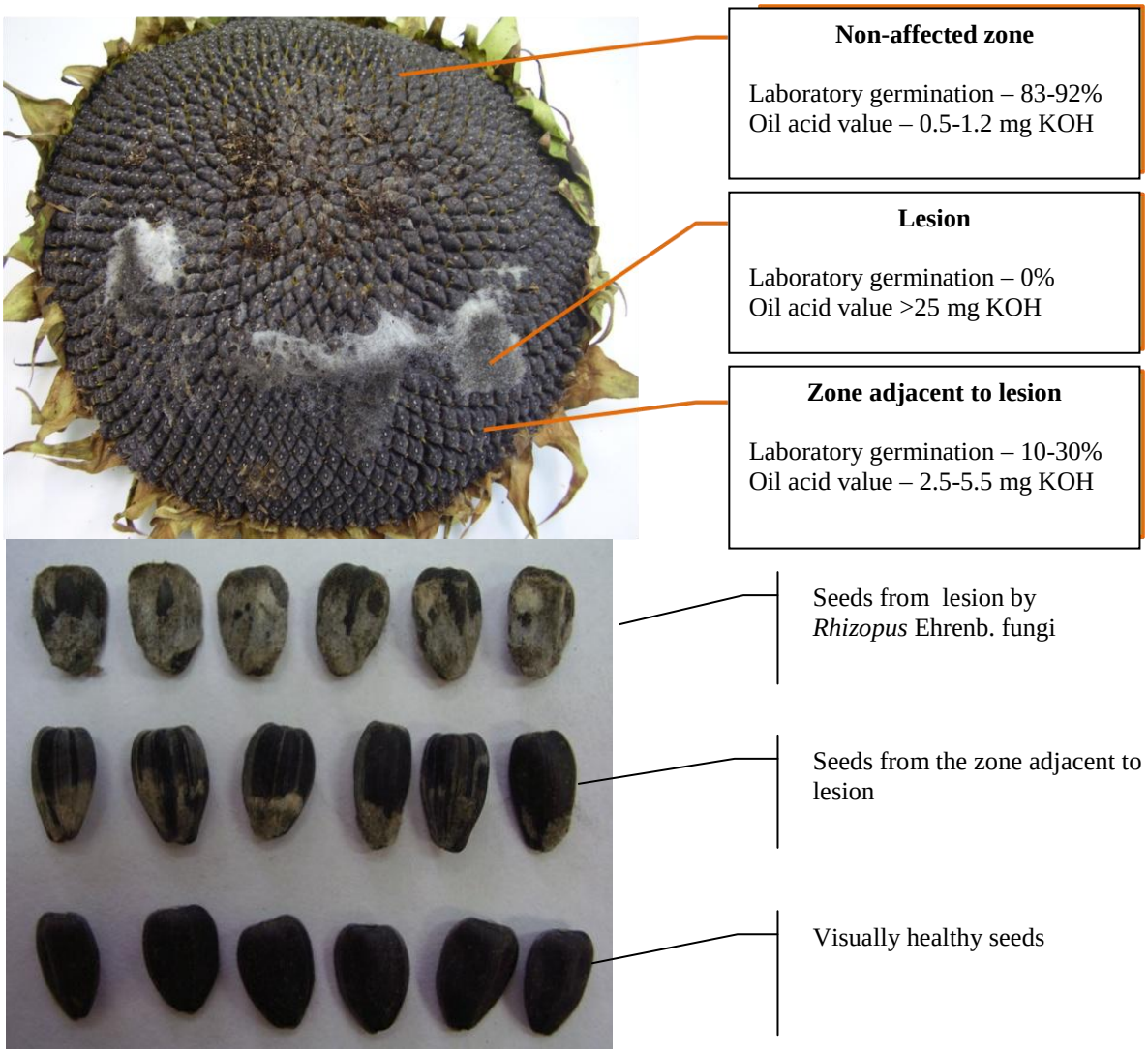

Fig. 2. Seeds from the sunflower head affected to a different extent by Rhizopus pathogen.

On the basis of our findings concerning changes of economic traits, sowing qualities of seeds and oil acid value depending on degree of affection of sunflower head by Rhizopus we have developed five-point immunological scale for the evaluation and selection of sunflower forms resistant to pathogen (Table 2).

Table 2. Scale for the evaluation of sunflower breeding material on resistance to Rhizopus
Krasnodar, VNIIMK, 2006-2008

Resistance, point	Affection of the head surface, %	Oil acid value, mg KOH	Degree of resistance (susceptibility)
4	0	0.8±0.2	R – resistant
3	1-25	1.2±0.1	
2	25-50	1.9±0.5	MR – mid-resistant
1	51-75	2.7±0.6	S – susceptible
0	75-100	10.2±5.4	VS – very susceptible

Plants which showed head affection area less than 25% and increase of oil acid value in 1.5 times in comparison with healthy seeds were rated as resistant. Plants with affection area less than 50% and increase of oil acid value in 2-2.5 times were considered as mid-resistant. Plants with 75-100% head affection and increase of oil acid value in 3 or more times comparing with healthy seeds were rated as susceptible and very susceptible.

Capability of the breeding material evaluation and determination of biotypes resistant to Rhizopus according to our immunological scale was checked on variety SPK. 60 sunflower heads were selected and divided into three groups according to the immunological scale: resistant, mid-resistant and susceptible. Then, oil acid value and oil content of derived seeds were determined under laboratory conditions. Obtained data correlate with the immunological performance (Table 3).

Table 3. Immunological performance of variety SPK samples
Krasnodar, VNIIMK, variety SPK, 2008.

Area of affected head surface,	Immunological performance	Oil acid value, mg KOH			Oil content of absolutely dry achenes, %		
%		min	max	medium	min	max	medium
< 25 %	R	0.6	0.9	0,7	40.6	46.3	43.0
< 50 %	MR	1.5	2.3	1.6	33.7	48.7	39.8
< 100 %	S	6.7	21.9	13.5	22.1	38.6	31.8

CONCLUSIONS

It is necessary to conduct evaluation of sunflower plants resistance to Rhizopus pathogen under conditions of large-scale development of pathogen. Acid value of seed oil can serve as an indirect quantitative index of degree of resistance to *Rhizopus* Ehrenb. fungi by the absence of seed affection by any other fungi. Parameter of laboratory germination cannot be used as an indirect trait for the differentiation of breeding material on resistance to *Rhizopus* Ehrenb. fungi. This trait depends on many factors and is not inherited. Scale of evaluation developed by us makes it possible to evaluate and identify sunflower forms resistant to this pathogen on the basis of head's surface affection degree and acid value parameter.

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Screening of sunflower inbred lines to resistance to white rot on stalk

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ABSTRACT

Twenty two sunflower (*Helianthus annuus* L.) inbred lines were screened for resistance to white rot on stalk. Plants were inoculated at the budding stage, with 4-days old *Sclerotinia* mycelium grown on PDA medium. Mycelium was placed on the leaf top and covered with tin foil, and the leaf was put into transparent nylon bag in order to achieve high humidity. Spot length on leaf was measured 30 days after inoculation and plant resistance determined at the full flowering stage. Obtained results were analyzed by variance analysis and correlations between observed parameters were calculated.

Key words: resistance – sunflower – white rot

Study of heat shock protein expression in three isogonics sunflower hybrids

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ABSTRACT

The present research reports the finding of a small heat shock protein (sHSP) of 5.6 kDa as the protein induced by heat stress. Three different sunflower hybrids derived from the cross between a Cms, HA 89, and three different restorer inbred lines, with resistant, susceptible and normal reaction to drought, were cultivated in two different geographical areas, karaj, Iran and Udine, Italy. The determination of total protein content, structural polypeptide fractions of seed storage protein (SSP) concentration, and the electrophoresis analysis of proteins by means of lab-on-chip capillary electrophoresis had been carried out in order to evaluate the differences between polypeptides of the hybrids SSP. Fatty acid biosynthesis in relationship with the localities of cultivation was followed up, as well. It was determined that protein accumulation in achene occurs at a greater rate during the achene filling phase, in precision 9-25 days after pollination in all examined hybrids. Instead, the expression of sHSP has been found in only one hybrid derived from a restorer tolerant to drought cultivated in the hot-dry climate which expressed a polypeptide band of 5.6 kDa.

Key words: hybrid – sunflower – small heat shock protein (sHSP)

INTRODUCTION

Small heat shock proteins (sHsps) are produced ubiquitously in prokaryotic and eukaryotic cells upon heat. However, the production of high levels of heat shock proteins can also be triggered by exposure to different kinds of environmental stress conditions, such as infection, inflammation, exercise, exposure of the cell to toxins (ethanol, arsenic, trace metals and ultraviolet light), starvation, hypoxia (oxygen deprivation), nitrogen deficiency, or water deprivation (Puigderrajols et al., 2002).

The special importance of sHSPs in plants is suggested by unusual abundance and diversity. Six classes of sHSPs have been identified in plants based on their intracellular localization and sequence relatedness. Specific sHSPs, the cytosolic class I and class II proteins are also expressed in the absence of stress in maturing seeds of several species, and a role for these proteins in desiccation tolerance, dormancy, or germination has been hypothesized (Efeoglu, 2009). In any case, the heat shock proteins are referred to as stress proteins and their upregulation is sometimes described more generally as part of the stress response (Santoro, 2000). The increase in expression is transcriptionally regulated. The dramatic upregulation of the heat shock proteins is a key part of the heat shock response and is induced primarily by heat shock factor (Wu, 1995). The mechanism by which heat-shock (or other environmental stressors) activates the heat shock factor has not been determined. However, some studies suggest that an increase in damaged or abnormal proteins brings HSPs into action. HSPs are part of a group of proteins induced by environmental stress either to protect the plant from damage caused by the stress or to help repair the damage caused by the stress. There is very likely to be some overlap in function among the different stress proteins. In agreement with this assumption is the observation that one stress can induce protection against another (Lurie et al., 1994; Leshem and Kuiper, 1996).

Evolutionary analysis suggests that the sHSP gene families arose by gene duplication and divergence prior to the radiation of angiosperms. In general, the sHSPs are not found in normal vegetative tissues, but accumulate to high levels in response to heat stress (Puigderrajols et al., 2002). Specific sHSPs are also

expressed during various phases of plant development as part of the endogenous developmental programme. Thus, although the sHSPs are apparently not essential for basal cell functions as are the high molecular weight HSPs such as HSP90, HSP70 and HSP60, their functions are likely to be critical for survival and recovery from heat stress as well as for specific developmental processes (Wang et al., 2004). Biochemical analysis indicates that sHSPs are found in high molecular weight complexes between 200–400 kDa that are most likely composed solely of multiple sHSP subunits. Purified recombinant plant sHSPs facilitate reactivation of chemically denatured enzymes in a nucleotide-independent fashion and also prevent heat-induced aggregation or reverse inactivation of protein substrates. Therefore, it is suggested that sHSPs act in vivo as a type of molecular chaperone to bind partially denatured proteins preventing irreversible protein inactivation and aggregation, and that sHSP chaperone activity contributes to the development of thermotolerance (Lavoie et al., 1993, Plesofsky-Vig and Brambl, 1995).

In this work, we are aim to follow up the protein and fatty acid profile of the sunflower seeds during the seed maturation in the hybrids, in the relationship with the climate they were grown up.

In this regard, protein analysis has been carried out by means of lab-on-chip capillary electrophoresis in order to evaluate the differences between polypeptides of the hybrids SSP and the formation of new polypeptids related to the effect of climate changing. Fatty acid analysis has been performed through Gas Chromatography, as well.

MATERIALS AND METHODS

Plant materials:

Three sunflower hybrids derived from the crosses between three different restorers (13 S as drought susceptible, 28 R as drought resistant, and Ac 4122 as normal) and one female line (HA 89, Reg. no. GS-39, PI 642062). Inbred line 28 R has derived from the cross between *H. argophyllus* x *H. annuus* (De Romano and Vázquez, 2003). The materials have been generated in National Institute of Genetics and Biotechnology, NIGEB, and university of Udine.

To study the response of the sunflower hybrids against two different cultivation climate, karaj, Iran and Udine, North East Italy, under irrigated conditions. The experiments were laid in a randomized complete block design with four replicates with an individual plot size of 279 m^2 (9 x 31 m).

Four irrigations (30 mm each) were done for both localities, some urgent irrigation were done in Karaj after flowering.

Temperature media in Karaj and Udine, after flowering stage between mid June to mid July was 42±1ºC and 30±1ºC, respectively.

Soluble protein extraction from seeds:

Total sunflower seed proteins were extracted from 50 mg seed in 0.4 ml of 2 M urea 15 % glycerol, 0.1 M DDT and 0.1 M Tris/HCl, pH 8.8, using an ultra-sonic water bath for 15 minutes. Extracts were centrifuged at 11000 g for 5 minutes (Wang et al., 2007).

Protein analysis on Agilent Bioanalyzer:

A final extraction volume of 400 µl was selected as optimal, based on the resolution and intensities of peaks reported by the Agilent software. Each clarified extract (4 µl) was mixed with 2 µl of Agilent sample buffer and 84 µl of deionised water. This mixture (6 µl) was applied to one of the 10 sample wells on the Agilent Lab Chip (www.agilent.com/chem/labonachip).

Analysis of proteins in an Agilent 2100 Bioanalyzer Technologies carried out with a Protein 80 + chip. Each sample contained an internal standard comprising an upper marker of 200 kDa and a lower marker of 4.6 kDa. Chip included a ladder comprising reference proteins of 14.4, 21.4, 35.3, 45, 66.2, 97.4, 116.3 kDa, against which protein mobilities were compared for each analysis.

By means of the chip, size, purity and concentration information of the protein samples had daily identified, after flowering date till the end of achene maturation.

Preparing the Gel-Dye Mix, Destaining Solution and Denaturing Solution

After tempering the Protein 80 Dye Concentrate (blue) and the Protein 80 Gel-Matrix (red), a centrifuge (2500 g) stage and vortexing did carry out to obtain a uniform dye. The same procedure had carried out with destaining solution. 3.5 Vol-% of 1 M Dithiothreitol or ß-mercaptoethanol is prepared as buffer.

Preparing the Samples and the Ladder

4 µl protein sample was added to 2 µl denaturing solution, this mixture and aliquot of Protein 80 Ladder heated in water bath at 95 °C for 5 min. after cooling down, 84 µl deionized water was added to sample

and ladder. Then Ladder and Sample was loaded in the chip wells and placed in the Agilent 2100 bioanalyzer.

Fatty acid profile determination:

Methyl ester preparation for gas chromatography technique for reserve seeds were done as described by Conte *et al*. (1986). The total fatty acid composition was determined with a HRGC Mega 2, Fisons gas chromatography equipped with a split injection system and flame ionization detector (FID) a fused-silica capillary column 30m × 0.32mm i.d. and the percentages of fatty acids were obtained by integrating the peak with Chrom-Card, Fisons Ins. Software.

Experimental design and statistical analysis:

The experiment was carried out following a bifactorial complete randomized block design with three replicates with four plants for each replication. The first factor, genotypes, was constituted by the three hybrids, and the second factor, locality with two treatments, Karaj and Udine. We conducted statistical analyses of triplicate determinations of total protein content and fatty acid composition of considered genotypes by ANOVA. Significant differences were expressed as $P<0.05$, and the least significant difference procedure was used to compare means of genotypes.

RESULTS AND DISCUSSION

Sunflower seed is a significant source of proteins and a number of authors have studied correlations between the protein content and other seed characteristics (Joksimović, 1999). The seed protein could be modified for the genetic background of the seed and environment variation.

The results of total protein content assay of the achene in different hybrids cultivated in two localities, Karaj and Udine, are provided in figure 1.

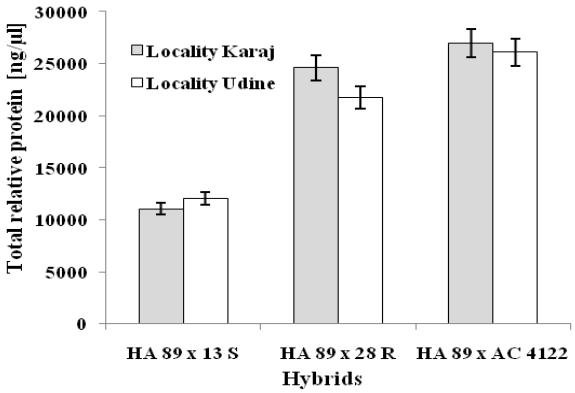

Fig. 1. A comparison of total protein concentration between different sunflower hybrids' achene cultivated in two localities, Karaj and Udine

Table 1. Statistic analysis of the differences in total relative protein concentration of three sunflower hybrids in two localities, Karaj and Udine

Hybrid	Total relative protein [ng/µL]	Locality	Total relative protein [ng/µL]
HA 89 x 13 S	11559.34 b	**Karaj**	21210.96 a
HA 89 x 28 R	23164.97 a	**Udine**	19618.87 a
HA 89 x AC 4122	26520.42 a		
LSD 0.05	4115.78	LSD 0.05	17840.60

Values with the same letter in the same column were not significantly different ($p<0.05$)

Means followed by the same letter are not significantly different at 1% level as indicated by Student–Newman-Keuls Test.

Higher amount of protein synthesis accumulation was registered in the hybrids cultivated in Karaj. It should be taken in consideration that in the phase of maturation the biosynthesis of proteins or oil is completely affected by environment besides genotypes (Merrien *et al*., 1988). Environmental factors affect certain physiological processes within the seed during seed formation and filling.

Two hybrids derived from the crosses HA 89 x 13 S and HA 89 x 28 R were provided higher protein concentration and no significant analysis is recorded for the aforementioned hybrids cultivated either in Karaj or in Udine (Table 1).

The protein synthesis and accumulation in different hybrids in two localities, Karaj and Udine after achene formation till achene maturation is measured daily and the data are reported in percent in figure 2. The ongoing increase of protein concentration during the achene maturation establishes the fact in which the biosynthesis of proteins in sunflower achene is a phenomenon happens in the maturation phase of achene (Flagella, 2006).

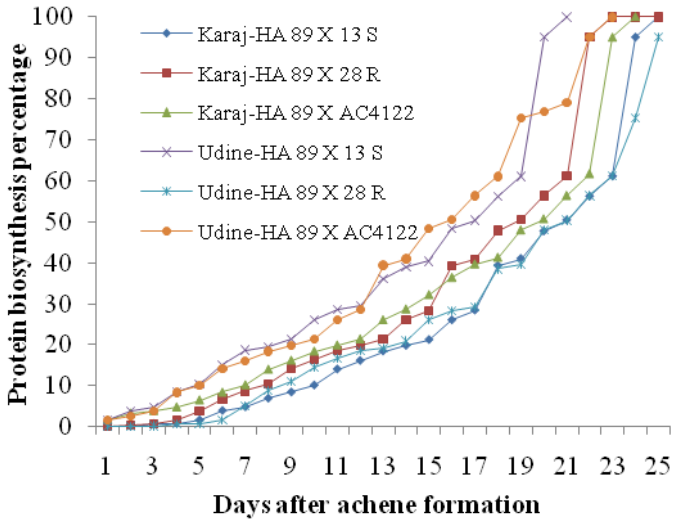

Fig. 2. Study of the protein accumulation in different hybrids in two localities, Karaj and Udine

Another parameter which could be probably the responsible for altering protein content in different localities is the nitrogen content of the soil (Dordas and Sioulas, 2008). As it shown in table 2, both localities are nitrogen deficient and it is to deduct the total protein content was not affected by the nitrogen content of the experimental field soil.

Table 2. Chemical properties of experimental field soil

Component	**Karaj**	**Udine**
Sand *g/kg*	700	400
silt *g/kg*	153	430
clay *g/kg*	80	170
nitrogen *g/kg (N)*	0.1	0.2
Phosphorus *mg/kg (P)*	15	45
Potash *mg/kg (K)*	327	200

Figure 3 demonstrates a Lab-on-a-Chip virtual gel-like electrophoresis of proteins extracted from the achenes after anthesis of different sunflower hybrids cultivated in two localities karaj, Iran. The virtual gel is a type of capillary electrophoresis which provides banding pattern of the sample's polypeptides against the ladder.

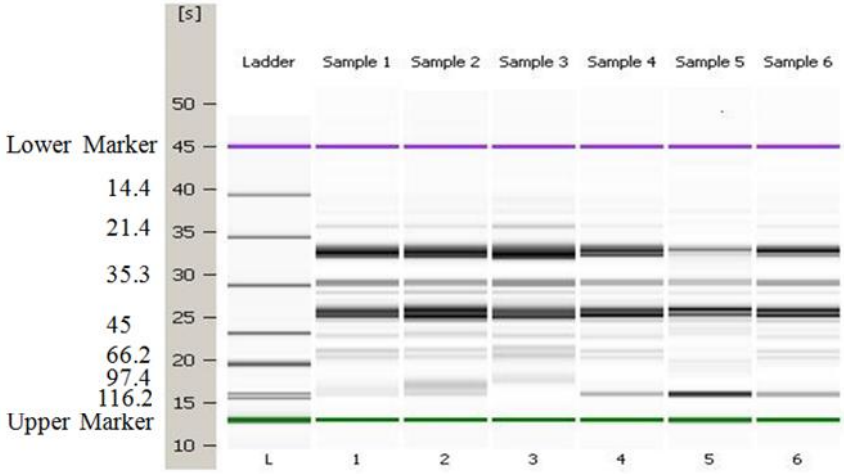

Fig. 3. Lab-on-a-Chip virtual gel-like electrophoresis of total proteins extracted from achene 9-25 DAP of three sunflower hybrids, cultivated in two localities karaj (samples 1, 2, 3) and Udine (samples 4, 5, 6). Molecular weight of bands of Ladder is expressed in kDa.

The presence and concentrations of polypeptides of different molecular weight is reported in Table 3. The polypeptide profile demonstrates the expression of polypeptides with the same molecular weight in either one locality or both localities. The only polypeptide that was expressed in Karaj locality with 5.6 kDa marked by a round circle implies with the expression of this small polypeptide as the consequence of higher temperature in Karaj which triggered the synthesis of small heat shock protein. It is notable that this sHSP has synthesised in the hybrid derived from the cross between HA 89 x 28 R that confirms the drought resistance of the restorer line, 28 R (de Romano and Vázquez, 2003).

Table 3. Concentration of polypeptide profile of total protein extracted from different genotypes cultivated in two localities, Karaj and Udine

	Molecular weight (kDa) / **Hybrids**	**200**	**116.3**	**72.31**	**66.2**	**45**	**33.5**	**27.3**	**21**	**6.6**	**5.6**	**4.6**
		Protein concentration [ng/µl]										
Karaj	HA 89 x 13 S	555.8			2084.4	2224.1	259.7	1363.8		3782.9		156.1
	HA 89 x 28 R	1731.7		4196.1	5001.6	473.4	3109.6		8122.1		412.6	
	HA 89 x Ac 4122	2359.4		5352.2	5249.6	581.2	4069.7		7060.9			275.3
Udine	HA 89 x 13 S		514		2103.7	2249.4	241.1	1371.5		2871.4		145.9
	HA 89 x 28 R		1866.8		4810.8	4219.8	501.2	3258.3		4205.9		128.4
	HA 89 x Ac 4122		1230.6		4376.6	4445.9	920.7	4858.8		4467.4		188.2

Besides protein accumulation that occurs in the achene maturation phase, oil synthesis and accumulation happens in that stage. There is a certain degree of antagonism between oil and protein biosynthesis in seeds of oil plants. Radic et al., (2009) found that in the process of maturing, protein biosynthesis stabilizes earlier than oil biosynthesis in sunflower seed.

Škorić *et al*. (1990, 1996) and mentioned that variations of oil content in sunflower seed in different years and localities, as well. Studying sunflower production in many localities, Kovačik *et al*. (1988) determined that environmental factors significantly affect oil content in seed.

The increase of oil content in sunflower seed can be achieved by gaining knowledge of environmental factors, application of adequate agrotechnical measures and correct choice of sunflower hybrids (Rabiei et al., 2008).

Table 4 is reported the statistical analysis of fatty acid biosynthesis in the achene maturation phase. It was observed that there is a significant difference between hybrids in the synthesis of oleic and linoleic

acid. As all studied hybrids have the same female line, this altering could be the effect of either restorer line or environment conditions.

Table 4. Statistic analysis of the differences in fatty acids synthesis of three different sunflower hybrids during achene maturation

Hybrid	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)
HA 89 x 13 S	6,47 a	4,66 a	28,89 b	59.93 ab
HA 89 x 28 R	6,33 a	4,13 a	32,64 a	56.78 b
HA 89 x AC4122	6,06 a	4,93 a	27.23 b	61.73 a
LSD 0.05	0.59	0.96	3.08	3.72

Values with the same letter in the same column were not significantly different ($p<0.05$)

Means followed by the same letter are not significantly different at 1% level as indicated by Student–Newman-Keuls Test.

However, in Table 5, the fatty acid modification of three hybrids was considered in the relationship of the localities they were cultivated.

Table 5. Statistic analysis of the differences in fatty acids synthesis during achene maturation in two localities, Karaj and Udine

Treatment	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)
Karaj, Iran	6,13 a	5,14 a	34.31 a	54.35 b
Udine, Italy	6,44 a	4,01 b	24,82 b	64.62 a
LSD 0.05	0.48	0.78	2.51	3.04

Values with the same letter in the same column were not significantly different ($p<0.05$)

Means followed by the same letter are not significantly different at 1% level as indicated by Student–Newman-Keuls Test.

Oleic and linoleic acid had shown a significant difference between hybrids when they are cultivated in two different localities. Elevated temperatures, and in particularly high night temperatures, caused a marked reduction in the percentage of linoleic acid, apparently due to the effect of temperature on the activity of the desaturase enzymes which are responsible for the conversion of oleic to linoleic acid (Harris et al., 1978).

CONCLUSION

As plants cannot move away from heat, they would have evolved a battery of specialized "stress genes" the sHSPs. These are expressed in response to heat in all subcellular compartments and could allow plants to cope better with the stress conditions on site.

The biosynthesis of a new polypeptide in only hybrid of the cross between HA 89 X 28 R against other hybrids cultivated in Karaj confirms the restorer role in demonstration of drought resistance. R 28 is derived from the cross between *H. annuus* and *H. agrophillus*, the latter is considered as gene pool of resistance against biotic and abiotic stress.

The impact of high temperature on fatty acid biosynthesis and accumulation is measured and confirmed that these modifications has a greater effect on unsaturated fatty acids rather saturated ones.

These results support the hypothesis that elevated temperature of 42ºC alters composition of sunflower protein and oil due to the effects of heat stress on the biosynthesis of sHSPs and fatty acids.

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Antioxidant activity changes in downy mildew infected sunflower triggered by BTH

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ABSTRACT

Although downy mildew of sunflower (Plasmopara halstedii) can be effectively controlled by using genetic resistant cultivars and seed treatment with fungicides, protection can be hindered by the genetic variability of this oomycete. Therefore, beside the traditional control strategies there was a need of looking for alternative methods to provide effective protection of this crop. One of the possible solutions might be the use of systemic induced resistance by applying the sunflowers with plant activators, like benzothiadiazole (BTH).

Earlier studies showed that BTH significantly reduced the appearance of disease symptoms (sporulation, stunting) in the infected plants. In the present work, we examined the activity changes of two antioxidant enzymes (polyphenol-oxidase, PPO and peroxidase, POX) in different sunflower lines following BTH pre-treatment and downy mildew inoculation.

The USDA sunflower inbred lines RHA 274, RHA 340 and HA 335, as well as the P. halstedii pathotype 700 were used to get one compatible, and two incompatible combinations, respectively. While HA 335 is characterized by complete resistance and RHA 340 with HLI (hypocotyl-limited) type partial resistance, the line RHA 274 is susceptible to pathotype 700. Enzyme activity assays were carried out using the spectrophotometric methodology.

P. halstedii inoculation significantly increased the enzyme activities both in susceptible and resistant lines but activities increased more rapidly and reached a higher value with time in the two resistant sunflower lines as compared to the susceptible one, with no respect of treatments. BTH enhanced the POX and PPO activity in the susceptible and completely resistant lines, either inoculated or not. However, the results of BTH treatment on sunflowers of the HLI type resistance were contradictory.

We assume that significant changes in enzyme activities shown in this study are in a good correlation with the enhanced host defense of sunflowers upon downy mildew attack.

Key words: benzothiadiazole – peroxidase – polyphenoloxidase – Plasmopara halstedii – SAR – sunflower

Phenol content in sunflower inbred lines infected with *Sclerotinia sclerotiorum*

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ABSTRACT

Eleven sunflower inbred lines were screened for resistance to *Sclerotinia*. Plants were inoculated with sclerotia. Stem was inoculated by direct sclerotium insertion at the beginning of the budding stage, and head by sclerotioum insertion into the back of the capitulum. Trial was set as completely randomized block system in three repetitions. Resistance to both forms of white rot was determined at the stage of the physiological maturitu, as the percentage of the healthy plants, as well as Mc Kinney disease index. Samples for phenol content determination were taken two weeks after inoculation. Differences between lines were detected considering disease incidence and severity, and phenol content.

Key words: phenol content – *Sclerotinia sclerotiorum* – sunflower

Evolution of the pathogen-host plant relationship, into the *Plasmopara halstedii* F. Berl. and de Toni- *Helianthus annuus* L. system, in Romania

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ABSTRACT

Romania is an important producing country of Europe with about 900.000 hectare/year and over 1.3 millions tones harvested seed. Downy Mildew induced by *Plasmopara helianthi*, is one of the limiting factors to sunflower production causing great yield damage up to the 30 % in favourable conditions for this pathogen. (Maria Pacureanu Joita et al., 2002).

The international set of differentials for the *Plasmopara halstedii* pathogen races, proposed by Gulya et al. 1991, together with Romanian ones, have been studied every year at the NARDI Fundulea under both natural and artficial inoculation. Isolates of this pathogen, proceeded from different Romania areas cultivated with sunflower have been tested.

Beginning with 2000 year, the physiological race spectrum of this pathogen which produces the Downy Mildew, changed. The differentials with resistance genes Pl_2 and Pl_5, are not resisting vs. the new-appeared more virulent races. New races of *Plasmopara halstedii* have been identified in north-eastern and western Romania, respectively 330 and 730. These races are present in other countries from Europe and North America, too.

Resistance breeding to Downy Mildew has so far been concerned with either major gene resistance. Their use in breeding do require an effort of backcrossing into different lines. Taking into consideration the economic importance of this disease for sunflower crop in Romania, new varieties have to be resistant to all Romanian races of *Plasmopara*, so parental lines were backcrossed to introduce new genes.

The use of partial resistance in addition to major genes to obtain more durable control of sunflower Downy Mildew is discussed.

Key words: differentials – pathogen races – resistance – sunflower

INTRODUCTION

Downy mildew, the sunflower disease caused by the pathogen *Plasmopara halstedii* (Farl.) Berl.and de Toni is one of the most dangerous for this crop, over the world. However, this pathogen may be controlled by the resistant hybrids or the treatment with different fungicides, there are some factors which are involved in being difficult this disease controll: the variability inside of the pathogen (Gulya et al.,1998) and pathogen resistance or tolerance to the fungicides, as metalaxyl (Albourie et al.,1998; Molinero-Ruiz et al., 2000).

In the last twenty years, a big change has become evident in the *Plasmopara halstedii* populations, in almost all the countries which are cultivating sunflower. In Europe, an increasing number of the pathotypes, each with a distinct virulence structure, have been identified. In Romania, five pathotypes of the pathogen, have been identified, before 2007 year (Pacureanu et al., 2006), in the last three years, being identified other two.

Since fungal diversity of this kind has consequences in both disease epidemiology and breeding for resistance, there is a need to identify the virulence of the local fungal populations and to monitor the changes over the time.

The most detailed description of the global distribution of *Plasmopara halstedii* pathotypes has been compiled by Gulya (2007), which comprised as many as 35 pathotypes (races), considering the fact that in most sunflower producing countries, just 12 well distinguished virulence pathotypes exist.

The clasical methodology for testing resistance to this pathogen it needed a sunflower genotypes differentials set, for the pathogen races, giving the possibility to identify the presence of different races in different areas cultivated with sunflower.

Obtaining sunflower hybrids resistant to *Plasmopara halstedii* is very important. For this reason, the breeders are continuosly looking for sources for resistance (Streten et al., 2006). In the last years, Radwan et al. (2004) in France and Dussle et al. (2004) in Germany have obtained good results with PCR markers for the *Pl5/Pl8* locus from complete CC-NBS-LRR sequences.

In the last 3-4 years, some researchers (Korosi et al., 2007; Vear et al., 2006) conducted studies which demonstrated that induced resistance might be useful for improving downy mildew management.

MATERIALS AND METHODS

For the pathogen races identification, in sunflower crop in Romania, it has been used the international differentials set for the *Plasmopara* races. We have used the same, some sunflower lines with good agronomic traits, which have been introduced into conversion process for resistance to downy mildew. Some commercial sunflower hybrids, there have been tested for their resistance to *Plasmopara halstedii* identification.

For the pathogen races studying we have collected attacked sunflower plants, from different areas cultivated with sunflower over the country. The samples were kept into refrigerateur, being used for doing the artificial infection. The study of the hybrids resistance was done in the natural infection conditions, in three years: 2007, 2008 and 2009.

RESULTS AND DISCUSSION

In Romania, the pathogen *Plasmopara halstedii* has increased the virulence, specialy in the last ten years, in this period being identified five new races (fig.1). In a period of 35 years, in sunflower crop in Romania were present only two races of the pathogen, but, in ten years this pathogen has developed other 5 races.

Fig.1. The evolution of *Plasmopara halstedii* races, in Romania

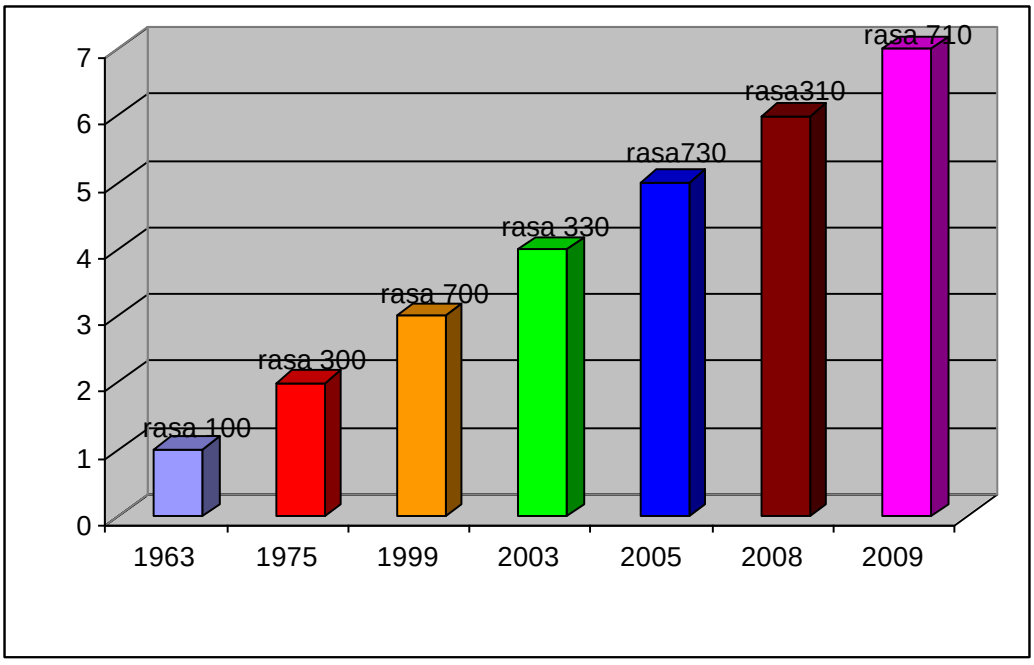


Table 1. Results of testing of the sunflower differentials set, for resistance to the pathogen (*Plasmopara halstedii*) races, Fundulea, 2008

Differentials	Izolates							
	Fundulea 1	Fundulea 2	Braila	Calarasi	Lovrin (Timiş)	Şimnic (Dolj)	Slobozia (Ialomiţa)	Valu lui Traian (Constanţa)
	Infection degree (%)							
Ad 66	64,7	48,4	49,7	44,3	58,9	43,2	39,4	55,7
HA-304	51,3	44,1	52,7	24,4	33,9	41,4	32,2	42,7
RHA-266	49,0	45,4	43,7	31,5	35,8	40,7	35,4	44,3
RHA-274	0,0	3,9	1,3	2,9	0,0	0,0	1,8	0,0

PMI 3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PM 17	0,0	0,0	1,9	0,0	3,8	1,3	0,0	0,0
803-1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
HAR 8	0,0	0,0	2,2	1,4	3,1	0,0	2,4	1,7
RHA 340	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
HA-335	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

For the pathogen races identification, we used eight isolates coming from seven areas cultivated with sunflower, over the country. It has been used two isolates from the same location (Fundulea), they being different. The results presented in table 1, are showing that in case of Fundulea 1 isolate, there are present only 100, 300 and 700 pathogen races, but in case of Fundulea 2 isolate, there are other two races: 310 and 330. The differential RHA 274 was attacked in other three cases: Braila, Calarasi and Slobozia isolates. There have infected the differentials: PM 17, in Braila, Lovrin and Simnic cases and HAR 8, for Braila, Calarasi, Lovrin, Slobozia and Constanta isolates.

Table 2. The patotypes of the pathogen *Plasmopara halstedii*, identified in the sunflower crop, in Romania

	Pathotypes							
Old name	**1**	**2**	**3**	**4**	**6**	**7**	**8**	**D**
New name / Location	**100**	**300**	**700**	**730**	**310**	**330**	**710**	**300**
Fundulea 1	**X**	**X**	**X**					**X**
Fundulea 2	**X**	**X**	**X**		**X**	**X**		**X**
Oradea (Bihor)	**X**	**X**			**X**	**X**		**X**
Podu Iloaiei (Iaşi)	**X**	**X**					**X**	**X**
Lovrin (Timiş)	**X**	**X**		**X**	**X**		**X**	**X**
Simnic (Craiova)	**X**	**X**					**X**	**X**
Slobozia (Ialomița)	**X**	**X**		**X**	**X**	**X**		**X**
Valu lui Traian (Constanța)	**X**	**X**		**X**				**X**
Braila	**X**	**X**		**X**			**X**	
Calaraşi	**X**	**X**		**X**	**X**			**X**

In table 2, we are presenting the *Plasmopara halstedii* pathotypes, in different areas in sunflower crop in Romania. So, the races 100 and 300 are present in all areas. The races 310 and 330 were identified in: Fundulea (one side of this area), Bihor, Ialomita, Calarasi. The race 310 was identified in Lovrin (Timis), Braila and Constanta, too. The race 710 is present in: Iasi, Timis, Craiova and Braila areas.

Table 3. Results of the improvment for resistance to downy mildew, for some sunflower genotypes, in Fundulea institute germplasm collection

CMS lines resistant to *Plasmopara halstedii*

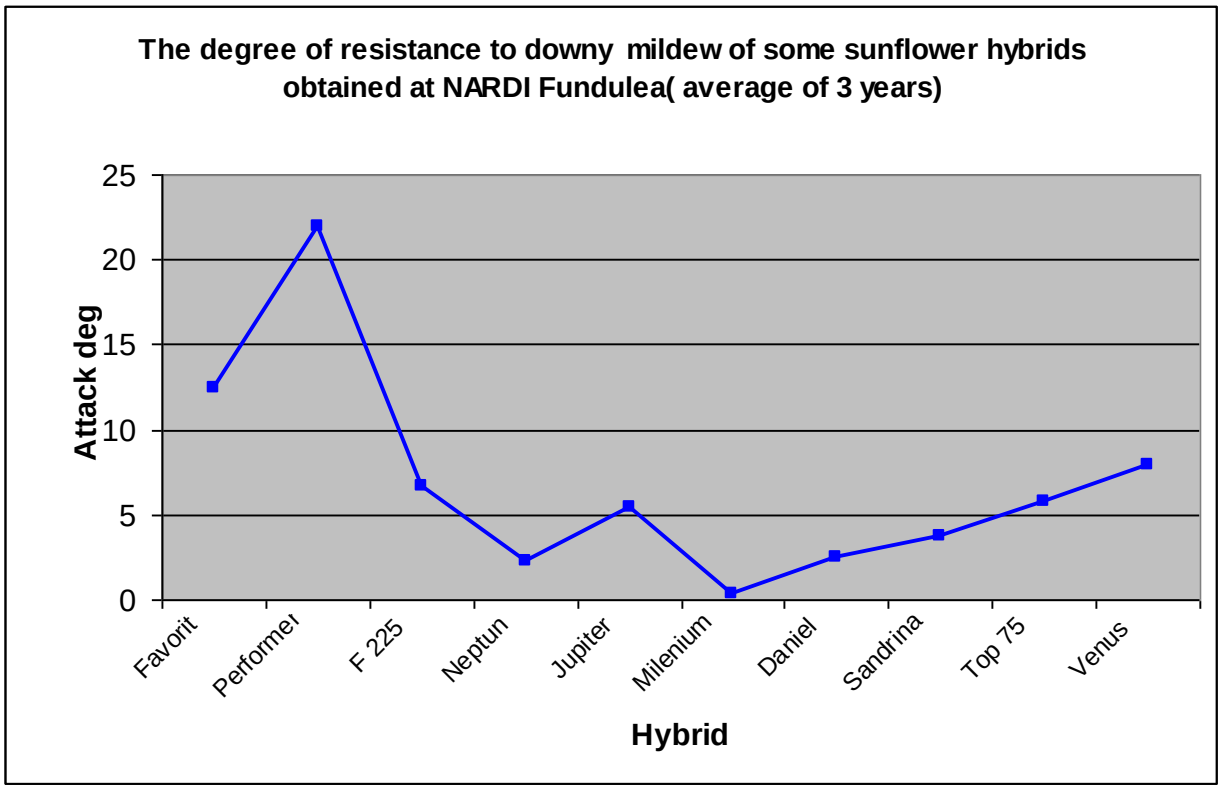
Source of resistance	Number of resistant lines
AS 110	31
RHA – 340	19
Populations	128

Restorer lines resistant to *Plasmopara halstedii*

Source of resistance	Number of resistant lines
SG – 861 b	27
HA – 335	4
Populations	189

Using different sources of resistance to the attack of *Plasmopara halstedii*, we have introduced different genes for resistance in some of our best sunflower inbred lines. The results are presented in table 3, the sources of resistance being used, depending on the line type: CMS or restorer lines. These lines will be used for the sunflower commercial hybrids obtaining, they being introduced into the seed market, only when the pathogen races will be spread in the large area cultivated with sunflower.

Fig.2



The sunflower hybrids obtained at Fundulea institute are resistant to different races of the pathogen, their behavior to downy mildew being presented in the fig. 2. It can be observed that for the new obtained hybrids, the degree of resistance, increased.

CONCLUSIONS

In sunflower crop in Romania, the pathogen *Plasmopara halstedii* has developed in last years new races. In a short period, of ten years, five new races of this pathogen it has been identified. The new races are present in western and southwestern Romania.

In the sunflower breeding activity at Fundulea institute we have obtained some resistant inbred lines, in order to obtain resistant commercial hybrids.

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Identification of physiological races of sunflower rust and reaction of the genotypes to the disease in Iran

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ABSTRACT

Sunflower rust in Iran is considered as an important specific disease for the crop. During years 2002 to 2004, northwestern and northeastern provinces, including West and East Azarbaijans, Ardebil, Semnan, Golestan and Khorasan, were surveyed on the distribution of the disease. Based on the surveys, two provinces, West Azarbaijan (Khoy area) and Golestan (Golidagh area), showed more disease severity than the others. Therefore, among 48 collected sunflower leaves showing rust symptoms, four samples were inoculated on susceptible variety Record and then purified throughout single pustule technique. Employing nine sunflower differential lines, the mass-produced isolates were differentiated in physiological races. The results obtained, showed that the isolates from West Azarbaijan and Golestan had reactions similar to races 300 and 302, respectively. Resistance of 16 sunflower inbred lines and 28 individuals of restorer line R-28 to representative race 300 was evaluated under controlled conditions. Eight individuals of R-28 and 13 inbred lines demonstrated highly resistant (HR) reaction against the disease.

Key words: sunflower – rust – physiological races – resistance – genotypes

INTRODUCTION

Sunflower rust disease caused by *Puccinia helianth*i was reported first time at 1822 on collected samples from Eastern south areas of United States (Sackston 1992). Incidence and severity of the disease on commercial hybrids had been increased up to early 1990s in Canada. Between 1988-89, the incidence on crop was reported up to 60 percent; whilst highly infected fields were appeared at the end of crop season (Rashid 1992). It is considered as an important disease at sunflower cropping areas of South and North America, Argentina, Africa, India, China, and Australia and has been reported on the host from all around the world (Lambrides and Miller 1994; Patil et al. 1998; Skoric 1998). According to Gulya and Masirevic (1995), *Puccinia helianthi* has worldwide distribution on sunflower and all species of *Helianthus*.

There are also four other species which cause rust on cultivated and wild sunflowers. *P. enceliae* and *P. masalis* have been reported on wild sunflower and composites from the south-west of United States. *P. xanthi* with worldwide distribution on *Xanthium* Spp from Australia and *Coleosporium helianthi* from eastern areas of U.S. have been reported (Gulya snd Masirevic 1995). Abbassi and Alizadeh (2000) in his investigations reported some areas of Mazandaran and Golestan provinces showing infection including Behshahr, Kalaleh, Golidagh, Kalpoosh plain and Gonbad. Sunflower rust in Iran is considered as one of the important diseases which cause economic losses every few years. For instance, it had 10 percent yield loss and 9.8 percent loss on oil yield (Abbassi and Alizadeh 2000).

Resistance resources to sunflower rust were found at 1949-1951 randomly in Canada (Sackston 1992). Genetic studies revealed that resistance is controlled by two dominant genes R_1 and R_2 (Skoric 1988). By these identified genes, four physiological races of the pathogen became determinable (Miller et al. 1992; Rashid 1992). The current determination happened based on three differential lines including CM-90RR, Cross 29-3 containing resistance genes R_1 and R_2 respectively and susceptible line S37-388. Therefore, races 1-4 were identified at early 1960 in Manitoba, Canada. The frequency of the races 1, 2, 3, and 4 were 62, 22, 16, and 2 percents, respectively (Rashid 1992; Lambrides 1994). Variation on races 1, 3, and 4 occurred with severed incidence on American differential lines. The results revealed that variation in pathogenicity of sunflower rust have been happened (Rashid 1992; Sackston 1992). The identification of four American races was possible at first step employing three differential lines and subsequently at least 25 differential lines were introduced which had mostly even reactions against the disease. Kong (1999) introduced 22 lines for identifying the races of sunflower rust. The lack of having complete and standard group as differential lines, encouraged Gulya and Masirevic (1994) to propose nine differential lines. Meanwhile, Lambrides and Miller (1994) used 13 lines to study pathogenicity of sunflower rust isolates.

Although, introduction of sunflower hybrids resistant to rust decreased the incidence but their long term presence ended to varied races which were pathogenic on resistant genotypes. In Australia, the race

3 was distributed on hybrids containing resistance gene R_1 (Rashid 1992). Rashid (1992) reported that the population of rust disease demonstrated strong increase on incidence and severity at Manitoba, Canada. The same observations were reported at North Dakota of United States (Rashid 1992). He employed Canadian lines CM-90RR, 29-3, and S37-388 and American lines HA-R1, HA-R2, HA-R3, HA-R4, and HA-R5.

The presence of several sunflower lines resistant to rust with different origins reveals genetic variation for resistance to the disease (Miller 1992). Resistant lines of sunflower to several races of rust have been bred and introduced. They have been derived from sunflowers native to Argentina, Yugoslavia and United States (Gulya et al. 2000). A large number of rust races especially 1-6 and several sub races have been identified at North America areas and field evaluations during 1990 determined that all commercial hybrids are susceptible to at least one race of rust disease (Rashid 1992; Lambrides and Miller 1994). Several genes expressing resistance to the rust disease have been identified in sunflower. Gene R_1 causes resistance to American races 1 and 2. Resistance to races 1 and 3, and 4 is controlled by genes R2, and R4 and R5, respectively. Whilst, gene Pu_6 controls resistance to races 1, 2, 3, and 4. Finally genes Ph_1, Ph_2, and Ph_{2a} composed with gene Ph_3 express resistance to race 340 from Argentina (Lambrides and Miller 1994). During 1988-1990, Rashid (1992) evaluated reaction of 185 sunflower commercial hybrids under natural conditions of infection. Scientists classified reaction types of sunflower germplasm into groups based on percentage and size of rust pustules. According their study zero was used for immune reactions and susceptibility with scale 4 was used for genotypes with uredial pustules showing diameter more than 0.6 mm (Patil et al. 1988; Rashid 1992; Lambrides and Miller 1994; Gulya and Miller 1995; Vicente and Zazzerini 1997). Gulya et al. (2000) employing coverage rate diagrams of rust pustules, classified reactions into immune (without pustule), highly resistant (0.1% leaf area), and susceptible (more than 0.5% leaf area) plants. Gulya and Masirevic (1995) used computer generated leaf diagrams to categorize sunflower genotypes based on percentages of leaf area covered with rust pustules. According their investigation, three reactions were considered for the plants including immune (without rust pustule), highly resistant (with up to 0.5% pustule coverage), and susceptible (more than 1% pustule coverage). Alizadeh and Abbassi (2001), evaluating diameter of rust pustules and their density, studied reaction of 54 sunflower genotypes. They used five categories in their investigations including immune, highly resistant, resistant, susceptible, and highly susceptible. 17 restorer lines of sunflower were categorized as highly resistant.

In this study, the variation of physiological races of sunflower rust was investigated to identify possible new races of the pathogen, *Puccinia helianthi*. To release and introduce new sunflower hybrids with resistance to rust disease, resistance of sunflower inbred lines and individuals of restorer line R-28 to representative race 300 was evaluated under controlled conditions.

MATERIALS AND METHODS

Collection and mass production of rust samples

Sunflower leaves showing developed pustules of rust were collected in dry paper bags and transferred to plant pathology lab in oilseed crops research department, Karaj. The collection areas from provinces are as followed (figure 1):

Table 1. Collection areas of sunflower rust during 2002-2004 from West Azerbayjan and Golestan provinces, Iran.

Province	Area	Host	No. of samples
West Azerbayjan	Khoy	Exp. Hybrid	1
		Confectionary Sun.	4
		Record	2
		CMS19	1
East Azerbayjan	Marand	Confectionary Sun.	2
Golestan	Golidagh	Record	7
		Progress	1
		Golshid	2
		Gabor	1
		Hysun33	1
		Hysun25	1
	Gonbad	Record	1
Khorasan	Golestan Jungle	Record	1
Ardebil	Moghan	Zaria	1
Semnan	Kaalpush Plain	Record	1

The collected inoculums were inoculated on susceptible variety Record immediately, under isolate conditions in greenhouse. Two or four leaf seedlings of the susceptible sunflower firstly were sprayed with distilled water to make the leaves wet. Using a piece of cotton swab, the urediospores were collected from the sample leaves and then rubbed gently onto the upper surface of leaves. The inoculated plants were covered with plastic bags to provide humid and dark conditions for 24 hours. The bags were removed and inoculated plants were incubated continuously to produce pustule on the leaves. After 10-15 days urediospores of a single pustule were used by the same method on two week old Record plants to produce purified isolates. Two isolates from each provinces West Azerbaijan and Golestan were provided as representative isolates because of restriction on greenhouse space and isolation (table 2).

Table 2. Sunflower rust isolates used for identification of physiological races

Isolate	Collcetion Area	Date	Host Genotype
Ph1312	Golidagh, Golestan	2001	Golshid
Ph1315	Golidagh, Golestan	2001	Record
Ph301	Khoy, West Azerbaijan	2002	Record
Ph302	Khoy, West Azerbaijan	2002	CMS19

Identification of physiological races

Nine Canadian and American sunflower differential lines introduced by Gulya and Masirevic (1995) were used to identify physiological races of *Puccinia helianthi*. These lines have been categorized into three groups which have different reaction pattern to the rust disease and are segregated pedigrees. Seeds of the lines after surface disinfection with 5% sodium hypochlorite were sown in pots containing pasteurized soil. Seedlings at V2 stage were sprayed with mix of distilled water and a small drop of Tween20 as emulsifier. Employing a smooth brush, the mixture of talc powder and urediospores (1000:1 g/g) was inoculated gently and then the plants were covered by plastic bags for 24 hours. After removing the plastic bags, the powder was washed up and removed from leaf surfaces. The inoculated plants were incubated for 14 days at greenhouse and then the reaction of differential lines to the inoculations was measured.

Evaluation of resistance to the disease

Resistance of 44 sunflower inbred lines and individuals of restorer line R-28 to representative race 300 was evaluated under controlled conditions. For this purpose, isolate Ph1301 identified as race number 300 (race 2 American) was mass-produced and used for the resistance evaluations. Inoculation and incubation methods were the same as for race identification procedure. The reaction of sunflower genotypes was evaluated by measuring pustule coverage percentage (PCP) according to computer-generated leaf diagrams depicting various percentages of leaf area covered with rust pustules (Gulya and Masirevic 1995). The method of interpreting resistance proposed by Gulya and Masirevic (1995) was used in which three reaction patterns are defined as followed: immune (PCP= 0%), highly resistant (0.5%>PCP>0%), and susceptible (PCP.1%).

RESULTS AND DISCUSSION

The all four isolates infected line S-37-388 (without any resistance gene) and demonstrated pathogenicity on sunflower. Isolates of West Azerbaijan induced similar reaction type on nine differential lines and comparing to original race designations (Gulya and Masirevic 1995), is categorized as race 2 American which is called race 300 based on numeric nomenclature. Line CM90RR carrying resistance gene R1 was showed susceptibility to the isolates. On the other hand, lines CM90RR and HA-R4 resulted in infection and spore production by Golestan isolates. Therefore, it was called race 302 according to numeric nomenclature (table 3). Abbasi and Alizadeh (2000) had identified race 3 of the pathogen in Golestan province and they had used four line set in their investigation. They had used lines S37-388, CM90RR, 29-3, and HA-R5 to determine the prevalent physiological race of sunflower rust. In the recent investigation, the designated race for Golestan province did not infect the line HA-R5 as well. Totally, the hot spots of sunflower rust disease in Iran are located in east and south-east of Golestan and center parts of West Azerbaijan. As the results show, the designated races of both provinces are close and induce same reactions on susceptible and other lines unless line HA-R4.

Table 3. Reactions and coded formulas of nine differential lines to nomenclature races and two newly designated Iranian races

	Original Race Designations											
Differentials	1	2	3	4	A	B	C	D	**Ph1312**	**Ph1315**	**Ph301**	**Ph302**
SET ONE												
S-37-388	1*	1	1	1	1	1	1	1	**1**	**1**	**1**	**1**
CM90RR	0**	2	0	2	0	2	2	2	**2**	**2**	**2**	**2**
MC29	0	0	4	4	0	0	0	4	**0**	**0**	**0**	**0**
SET TWO												
P-386	0	0	0	0	1	0	0	0	**0**	**0**	**0**	**0**
HA-R1	0	0	0	0	2	0	2	0	**0**	**0**	**0**	**0**
HA-R2	0	0	0	0	0	0	4	4	**0**	**0**	**0**	**0**
SET THREE												
HA-R3	0	0	0	0	0	1	0	1	**0**	**0**	**0**	**0**
HA-R4	0	0	0	0	0	0	0	2	**2**	**2**	**0**	**0**
HA-R5	0	0	0	0	0	4	4	4	**0**	**0**	**0**	**0**
Coded Virulence Formulas	100	300	500	700	130	305	364	747	**302**	**302**	**300**	**300**

* Values 1, 2, or 4 denotes a susceptible reaction ** value zero denotes a resistant reaction

Among 28 individuals of restorer line R-28 tested in this investigation, 2, 8, and 18 lines demonstrated immune, highly resistant, and susceptible reactions respectively (table 4). In contrast, major number of inbred lines of sunflower showed highly resistance against the disease (table 5).

Table 4. Reaction of sunflower individuals of restorer line R-28 to race 300 of *Puccinia helianthi*

Genotype	PCP (%)	Reaction	Genotype	PCP (%)	Reaction
R-28.1	2	S	R-28.2	0.5	HR
R-28.3	1	S	R-28.4	2	S
R-28.5	0.1	HR	R-28.6	2	S
R-28.7	2	S	R-28.8	1	S
R-28.9	2	S	R-28.10	0.5	HR
R-28.11	1	S	R-28.12	0.1	HR
R-28.13	0.1	HR	R-28.14	1	S
R-28.15	2	S	R-28.16	2	S
R-28.17	5	S	R-28.18	2	S
R-28.19	0.5	HR	R-28.20	0	IM
R-28.21	0.5	HR	R-28.22	0.5	HR
R-28.23	0	IM	R-28.24	1	S
R-28.25	2	S	R-28.26	10	S
R-28.27	2	S	R-28.28	1	S

Table 5. Reaction of sunflower inbred lines to race 300 of *Puccinia helianthi*

Genotype	PCP (%)	Reaction	Genotype	PCP (%)	Reaction
600.1	5	S	619.4	0.1	HR
603.4	5	S	621.2	0.5	HR
605.2	0.5	HR	640.1	0.1	HR
607.2	0.1	HR	641.2	0.1	HR
608.4	0.5	HR	650.3	0.1	HR
611.1	0.1	HR	651.1	0.1	HR
614.4	0.5	HR	652.4	0.1	HR
615.4	2	S	653.1	0.1	HR

Occurrence and distribution of physiological races of sunflower rust has importance in plant breeding research; as the process of breeding for resistant genotypes would be broken without considering the variation and distribution of the disease physiological races. In addition, genetic resistance to rust is controlled by dominant genes. Thus, access to new resistant sources in sunflower breeding is more likely. Among 44 lines and individuals of sunflower derived at the breeding processes, 23 resistant ones could help breeders to improve and release new hybrids or varieties containing resistance sources to the rust.

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Towards the characterization of a Quantitative Resistance to Downy Mildew in cultivated Sunflower, Helianthus annuus

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ABSTRACT

Quantitative resistance to sunflower Downy Mildew caused by the oomycete Plasmopara halstedii was studied on a population of recombinant inbred lines (RIL) not carrying efficient major resistance gene, in fields naturally infested by one race of the pathogen (703 or 710). The major quantitative trait locus (QTL) localized on linkage group 10 explains almost 40% of variation, and is not linked to any of the known race-specific resistance genes called Pl genes. This QTL confers resistance to at least 2 different downy mildew races and its support interval is 5 cM long. We constructed and screened a BAC library of the RIL parent (XRQ) having the QTL with the closest genetic markers in order to build a BAC contig in the QTL region, a first step towards the positional cloning strategy. The polymorphic BAC ends are currently being used as new genetic markers on the RIL population. We also screened an F2 population of 3500 plants in order to increase the number of plants presenting a recombination event between the closest QTL markers. The evaluation of the resistant phenotypes of such recombinant plants may help restricting the QTL support interval. In order to characterize the expressed genes during the interaction from both partners, plant and oomycete, we initiated a cDNA sequencing approach of infected sunflower plantlets using the 454® sequencing method.

Key words: Plasmopara halstedii – Helianthus annuus – QTL – quantitative resistance – cDNA sequencing

V. PROTECTION OF PLANTS AGAINST DISEASES AND BROOMRAPE

Effect of genotype, chemical treatment and storage conditions on sunflower germination energy

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ABSTRACT

Sunflower seed pathogens might greatly affect seed viability if it is not treated with suitable fungicides. Beside pathogens, pests also could endanger early development of sunflower plants. Because of that, seed is often submitted to treatment with insecticides.

Influence of chemical treatment on field emergence of commercial sunflower hybrids NS-H-111, Sremac and Šumadinac stored in different conditions, had been tested in 2007 and 2008 on experimental field of the Institute of Field and Vegetable Crops.

Based on experimental results, highly significant was influence of genotype and storage conditions, while influence of chemical treatment was significant. In addition to this, interactions between factors were not significant. In average, the highest value of field emergence had hybrid NS-H-111 (88.79%) and for chemical treatment fludioxonil + methalaxyl + imidacloprid (87.71%). Seed kept in common storage had the highest emergence value in field (87.92%). Highest field emergence had seed treated with fludioxonil + methalaxyl + imidacloprid and stored one year in common storage (90.18%). Considering interaction between storage conditions and genotype seed of hybrid NS-H-111 sown after chemical treatment had highest field emergence (91.82%) and seed kept in common storage slightly (90.48%). Seed of hybrid NS-H-111 compared with other two had highest field emergence treated with fludioxonil + methalaxyl + imidacloprid (91.84%).

Key words: sunflower seed – field emergence – genotype – chemical treatment – storage conditions

Biological peculiarities of infection keeping in sunflower seeds

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ABSTRACT

The research is dedicated to studying the inner mycoflora of sunflower seeds. Some biological peculiarities concerning keeping of pathogens causing epiphytotically dangerous diseases, such as Phomopsis, White Rot and Downy Mildew, are determined.

Key words: pericarp – embryo – infection – latent infection – symptoms – disease – head – stem.

INTRODUCTION

It is well-known that in addition to soil and plant residues seeds play a significant role in keeping sources of pathogens and expansion of disease area.

Sunflower seeds also serve as a substrate favorable for the development of various microflora, mainly of fungal one.

Sources of sunflower pathogens are localized in different organs and tissues of seeds, what causes the inner seed infection, including the latent one. There are some well-known methods for identification of all types of sunflower seed infection.

In our research priority is given to the determination of different forms of infection keeping in sunflower seeds what is predetermined by using our new methods which made it possible to identify species and quantitative composition of the pathogenic mycoflora of sunflower seeds and localize pathogens in embryo tissues.

MATERIALS AND METHODS

In our research we were studying inner infection taken from the pericarp tissues and embryo. Latent infection also refers to the inner infection, but it requires another approach to its study. Method established by N.A. Naumova (1960) [1] is generally used nowadays. According to this method infection of sunflower seeds is revealed by their germination in a moisture chamber at a constant temperature (24.0-25.0^{o}C) within five days. Modification of this method consisted in the separate examination of the mycoflora of seed coat and seed kernel and in the changes of temperature conditions and sample incubation period. Temperature conditions were adjusted for every pathogen taken separately.

For the needs of research there were selected sunflower seeds taken from the plants which showed symptoms of the epiphytotically dangerous diseases: Phomopsis, pathogens fungi *Phomopsis helianthi*, Munt-Cvet. et al. and *Phomopsis arctii* Lach.; White Rot, pathogen *Sclerotinia sclerotiorum* (Lib de Bary); Downy Mildew, pathogen fungus *Plasmopara helianthi* Novot.

By the Phomopsis affection seeds were taken from the plants which had disease symptoms on heads or on stems only. We acted similarly in the case of White Rot affection. Affection of sunflower seeds by the Downy Mildew pathogen was determined on plants affected by the second form and from the plants with symptoms of the fifth form of disease manifestation.

Determination of sunflower seeds affection by the Downy Mildew pathogen under laboratory conditions was carried out according to the method which essence is expressed through the creation of conditions favorable for the display of the disease symptoms on different organs of seedlings. Confirmation of presence of the *Plasmopara helianthi* inoculum was established during the microscopic examination of tissue sections carrying disease symptoms.

RESULTS AND DISCUSSION

There's no consensus concerning the transfer of the Phomopsis infection through seeds. Some researchers [3, 4] suppose that infected seeds can't be the source of infection because perithecia, the finished stage of fungus, are not formed on pericarp. Organs of asexual sporification – pycnidia with ß-conidia – cannot germinate and be the source of affection as they have a deformed nucleus.

Some researchers studying this problem have opposite views, because they have obtained ascomycetous sporification (*Diaporte*) on seeds and seedlings of sunflower under laboratory conditions.

Unlike our researchers many authors studying biological peculiarities of Phomopsis have used sunflower seeds only in case of the head affection.

Based on the research results, phytoexamination of achenes taken from the affected heads showed 100% affection of pericarps by pathogen, and the same one of embryos. Analysis of seed samples taken from the plants with stem affection at a degree of disease development of 3-4 points (a four-point scale) showed infection of pericarps – 29.5%, germs – 89.5%, and latent infection – 0.5% on hypocotyls of embryos.

Latent form of fungi presence can be discovered through the browning of hypocotile tissues or through the formation of constrictions on it. By the latent pathogenesis of Phomopsis on the affected hypocotiles emerge pycnidia, in which ß-spores (*Phomopsis helianthi*) or ß- and λ-spores (*Ph. Arctii*) are formed. Quantitatively compared, latent infection by *Phomopsis helianthi* was 5.7%, by *Ph. Arctii* – 0.3%.

On the embryos of seeds taken from the affected heads and stems there were obtained fungal perithecia on potato glucose agar after 28–30 days of cultivation in common laboratory conditions by the air temperature fluctuations from 13 to 25^{o}C under natural light illumination with light period from 8 to 16 hours.

In the course of investigating the duration of Phomopsis pathogen keeping in seeds we have found out that within nine months of keeping under the laboratory conditions the affection of seeds completely remains. After the 18-months term of keeping we can see natural recovery of seeds and absence of the Phomopsis pathogen.

Examination of seeds taken from sunflower plants affected by White Rot showed that by the middle stalk rot infection on pericarps was 2.0%, on embryos – 74.0%. There was no latent infection detected. Phytoexamination of seeds from the affected heads showed infection on pericarps in rate of 84.9%, on embryos – 61.0%. There was also no latent infection detected.

After nine months of keeping there were no infectious sources of the White Rot pathogen detected on the pericarps of seeds from the plants with affected stems; infection of embryos reduced by 49.0%, but there were discovered 28.0% of seedlings with symptoms of latent infection. Thus, there was a redistribution of the infectious source from the inner form into the latent one.

After nine months of storage of seeds from the heads affected by White Rot there was a slight reduction in quantity of infected pericarps and embryos, latent form occurred on 4.0% of hypocotiles.

Methods which make it possible to establish that the infectious source of the Downy Mildew pathogen fungus *Plasmopara helianthi* remains in sunflower seeds have been developed by N.S. Novotelnova (1966) [7] and O.I. Tichonov (1969) [8]. The basis of each method was growing of sunflower by using seeds of affected plants under field conditions which make soil-borne and aerogenic infection impossible.

Calculations of seed affection by *Plasmopara helianthi* were conducted in two stages. At the first stage calculation was held on the 7-days germs developed under the moisture chamber conditions. Quantity of plants with undeveloped radicles, necrotic traits on hypocotiles, necrosis on cotyledons was recorded. At the second stage visually healthy 7-days seedlings were put into the vessels with distilled water. In a 7–8 days term quantity of plants with symptoms of latent infection such as chlorotic spots on the front side of cotyledons was calculated. Hyphae of *Plasmopara helianthi* fungus were detected in the intercellular space during the microscopic examination of tissue sections in the necrosis and chlorosis areas. In each variant total quantity of achenes affected by Downy Mildew was calculated by summing up seedlings with disease symptoms as a result of two stages of test.

Mycelium of *Plasmopara helianthi* was detected on 25.8% of embryos, including 9.3% with latent infection in the process of analyzing achenes taken from plants with symptoms of the fifth form of Downy Mildew. Phytoexamination of achenes after nine months of storage didn't show any significant changes in their affection.

Presence of latent infection in all organs of seedlings within the limits from 30.0 till 42.0% was identified in the course of examination of seeds of sunflower showing the symptoms of the second form of Downy Mildew development.

Conclusions:

1. Sunflower seeds are real sources of keeping and spreading of infectious pathogens.

2. Sunflower seeds may be infected by two forms of Phomopsis: *Phomopsis helianthi* and *Phomopsis arctii*.

3. It is determined that 28–30 days period is a sufficient term for the formation of perithecia of *Phomopsis* fungi under the conditions of temperature fluctuations from 13 to 25^{o}C and natural light illumination.

4. Infectious source of the Phomopsis and White Rot pathogens penetrates into seeds not only in case of achene affection, but also by stem affection.

5. All examined pathogens have a latent form of seed infection.

6. Infectious source of all studied pathogens remains in seeds within nine months. After the 18-month term of keeping we can observe a natural recovery of seeds affected by *Phomopsis* and *Sclerotinia sclerotiorum* fungi.

7. It is established that infection keeping in seeds from the sunflower plants affected by the stem form of White Rot is supplemented with redistribution of infection form. Infection partially turns from the common inner form into the latent one. At the same time, total quantity of affected seeds does not change.

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PICTOR – a new fungicide against the major diseases in sunflower

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ABSTRACT

A number of fungal diseases may affect the yield potential of the sunflower crop during the vegetation period. To add additional means for highly efficient fungal disease control BASF SE has registered or is going to register in Europe a broad-spectrum fungicide with a high level of activity on major diseases under the trade name PICTOR®.

PICTOR® is formulated as a suspension concentrate and combines two highly active fungicidal substances, with different mode of action, being Boscalid and Dimoxystrobin. Boscalid is a pyridine carboxamide and inhibits the enzyme succinate dehydrogenase (SDH), also known as complex II in the mitochondrial electron transport chain (Kulka and von Schmeling 1995). Dimoxystrobin belongs to the QoI group of fungicides and the mode of action is the inhibition of mitochondrial respiration resulting from a blockage of the electron transport from ubihydroquinone to cytochrome c by means of a binding to the ubihydroquinone oxidation centre (Qo) of the cytochrome bc_1 complex (Complex III).

In exact field trials PICTOR® showed effective control of Phoma macdonaldii, Botrytis cinerea, Alternaria spp., Sclerotinia sclerotiorum and Phomopsis helianthi (= Diaporthe helianthi), all being diseases with potentially significant impact on yield..

The multi-year trial series confirmed that a PICTOR® treatment provided good disease control resulting in excellent yield response under various conditions. Even under very low disease pressure, PICTOR® did provide yield increase, difficult to be explained by disease control only.

Current registrations of PICTOR® in e.g. Hungary allow two applications per season, from 8 leaf stage (BBCH 18) until flowering (BBCH 65). The recommended dose rate is 0, 5 l/ha. PICTOR® should always be applied protective to control diseases most efficient, especially Sclerotinia sclerotiorum.

Key words: Boscalid – Dimoxystrobin – disease – fungicide – PICTOR® – sunflower