DEVELOPMENT OF SUNFLOWER HYBRIDS RESISTANT TO HERBICIDES

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ABSTRACT

The distinctions between 18 IMI-resistant experimental sunflower hybrids were studied on the characters seed yield, seed oil content, seed oil yield, plant height, head diameter and climate conditions influence applying two-factor analysis of variance. Hybrids plasticity was established by studying their reaction to the variable climatic conditions. It was determined that hybrids 1111A x 14R; 864A x 18R; 802A x 43R; 864 x 43R exceeded the mean standard on the characters seed yield and seed oil content with the highest statistical authenticity P=0,001. The studied hybrid combinations, treated with herbicides Pulsar 40, were affected equally by the climatic conditions in the respect of the character seed oil content. The strongest was the influence of environmental conditions related to the characters seed yield and plant height. The reaction of experimental hybrids to the pathogens *Plasmopara helianthi, Phomopsis helianthi, Phoma macdonaldii* and the parasite broomrape (*Orobanche cumana*) was studied with aim to establish the resistant ones.

Key words: sunflower hybrids, imidazolinone resistance, CLHA-PLUS, pathogenes resistance

INTRODUCTION

Over the last several years, the Bulgarian oilseeds industry has invested in expanding capacities, diversifying and adding value to some of the major field crops. This trend has been most pronounced with the sunflower crop. While until recently Bulgaria was a net exporter of sunflower seeds, lately the country has increased its crushing capacity. As a result of these developments, Bulgaria is on a trend to consume most of its sunflower crop and become a net exporter of processed products. The planting areas have increased in recent years because of higher profitability, low input requirements and better exporting possibilities, but higher rates of disease and pests have severely limited the sunflower production in some years. Significant results have been recently achieved in sunflower breeding for tolerance to imidazolinone (IMI) herbicides. Using IMI herbicide resistant hybrids gave farmers, the opportunity to control broadleaf weeds such wide spread *Xanthium*, *Cirsium* sp. Imidazolinone herbicides control a broad spectrum of grass and broadleaf weeds in imidazolinone-tolerant sunflower, including weeds that are closely related to the crop itself and the key parasitic weed broomrape (Tan *et al*., 2005).

An imidazolinone-tolerant wild sunflower population that was discovered in soybean field in Kansas, USA in 1996 (Al-Khatib et al. 1998) was used as a source for insertion of imidazolinone-tolerance gene into the first imidazolinone-tolerant lines (Al-Khatib and Miller 2000).Two lines (IMISUN-1 and IMISUN-2) were developed with resistance to imidazolinone herbicides (Al-Khatib and Miller 2000). This was followed by the release of public IMISUN lines in 1998. The development of resistant IMISUN sunflower hybrids depends on both inbred lines, having IMI-resistant genes, because this resistance was controlled by two genes: a mojor gene with semi dominant type of gene action (*Imr1*) and second gene (*Imr2*) with a modifier effect (Bruniard and Miller, 2001; Sala *et al*., 2012).

Sala *et al*. (2008a, b) reported for obtaining a new source of IMI resistance, CLHA-PLUS, developed by induced mutations (ethyl-methanesulfonate mutagenesis) and selection. *CLHA-PLUS* was different from *Imr1* at the molecular level and was controlled by expression of the partially dominant nuclear allele *AHAS1-3*. This *CLHA-PLUS* gene has higher IMI resistance than the *Imr1* and *Imr2* genes and higher oil content in sunflower hybrids. This was widely known as the Clearfield System, a trademark of BASF *Co* (Evci *et al.*, 2012; Skoric, 2012; Kaya *et al*., 2013; Kaya, 2014). In recent years, IMI-Plus sunflower hybrids have been developed in many countries.

The aim of this investigation was to test experimental IMI-Plus resistant sunflower hybrids, compare their seed yield, oil content with standards and choose those with highest exceeding.

MATERIAL AND METHODS

The investigation was carried out in 2016 and 2017. Four sterile mother lines 1111A, 864 A, 802 A, 564 A and nine restorers 14 R, 18 R, 43 R, 48 R, 50 R, 51 R, 53 R, 55 R, 56 R with *CLHA-PLUS* gene were used. They were the result of implementation of long-term research program for developing herbicides resistant CMS lines, restorers and hybrids at DAI applying different breeding methods. The parental lines were morphologically uniformed. They were characterized with very good combining ability and resistance to *Plasmopara helianthi*. The standards, included in this testing were Adagio CX, LG5661 CL. Hybrid plants were treated at phase 3-5 pair of true leaves with the herbicides - Pulsar 40 (120 ml/da). Hybrids were tested at the experimental breeding fields of DZI-General Toshevo in a randomized block method in two repetitions, as the area of each repetition was 20 m^2 (Barov and Shanin, 1965). Phenological characters, conformed to UPOV characteristics, were determined. The seed oil content was determined on the method of Rushkovskii (1957). Phytopathological evaluations of F1 hybrids were carried out in laboratory conditions and in artificial infection plot. Evaluation for resistance to downy mildew (*Plasmopara halstedii* Farl. Berleseet de Toni) was carried out on the method of Vear and Tourvieille (1987). Evaluation for resistance to grey spots on sunflower (*Phomopsis/Diaporthe helianthi* Munt.-Cvet. et al.) was carried out on the method of Encheva and Kiryakov (2002) in field conditions on artificial infection plot. Evaluation for resistance to black spots on sunflower (*Phoma macdonaldii* Boerema / *Phoma oleracea* var. *helianthi-tuberosi* Sacc) was carried out on the method of Fayralla i Maric (1981) in field conditions on artificial infection plot.

The obtained data were analyzed by ANOVA 3, a statistical tool, used to develop and confirm an explanation for the obtained experimental data**.** The ANOVA F-test is known to be nearly optimal in the sense of minimizing false negative errors for a fixed rate of false positive errors. The two-way analysis of variance was applied. It examines the influence of two different categorical independent variables on one continuous dependent variable. The two-way ANOVA not only aims at assessing the main effect of each independent variable, but also, if there is any interaction between them. These two factors are YEAR (Y) and HYBRID (H).

RESULTS AND DISCUSSION

Rising temperature and altered soil moisture due to climate change is believed to decrease the yield of food crops over next 50 years. Drought is one of the environmental factors, limiting plant growth and the productivity of many crops. Sunflower is considered as comparatively drought tolerant crop. It has the ability to extract water from deeper in the soil profile than other crops, and thus it can extract more water from each soil layer. The period of investigation 2016-2017 was covered by difference on soil moisture supply. For the aim of the study, some meteorological data were summarized and analyzed (fig.1). The meteorological conditions during the vegetation period of sunflower were characterized by quantities of rainfall (mm) for the period April-October and average diurnal temperature range for that period. The agricultural and meteorological conditions were variable and that affected the plants growth. The analysis of the obtained data showed that the experimental hybrids were tolerant to both low and high temperatures, but more tolerant to low temperatures. A critical time for water stress was the period 20 days before and 20 days after flowering.

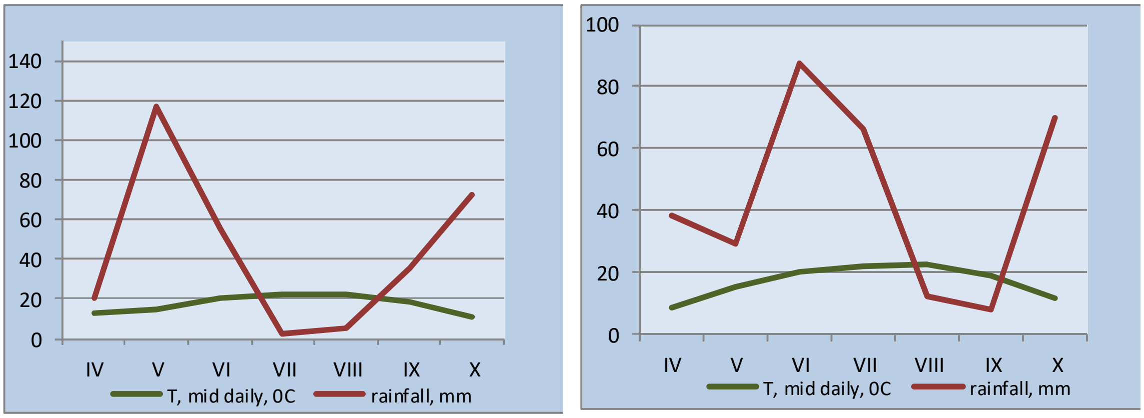

Fig.1. Temperature range (^{0}C) and rainfall (mm) during 2016-2017.

The summer weather in 2016 was unusually hot and dry for July and August (fig.1). Despite very good soil moisture reserves until middle of June, the scorching temperatures had a negative impact on sunflower yields. It also affected sunflower quality (oil content and the size of seeds). There have been above-average temperatures and scarce rainfall between mid- June and mid-August, which decreased the soil moisture content to below average levels, resulting in a 50-70 mm rainfall deficit as compared to the long-term average. Timely rains and moderate temperatures in early fall stimulated optimal planting in 2017. The summer weather remained dry and warm. Rainfall has been scarce since mid-August and September. Rains at the end of May and early July 2017 were very favorable for the crop.

Sunflower is very responsive to rainfall received during June and into September - starting just prior to flowering and continuing through seed fill. The seed yield was strongly affected when water deficiency appeared during flowering and in the following stages. A critical time for water stress was the period from beginning of button formation until the end of flowering.

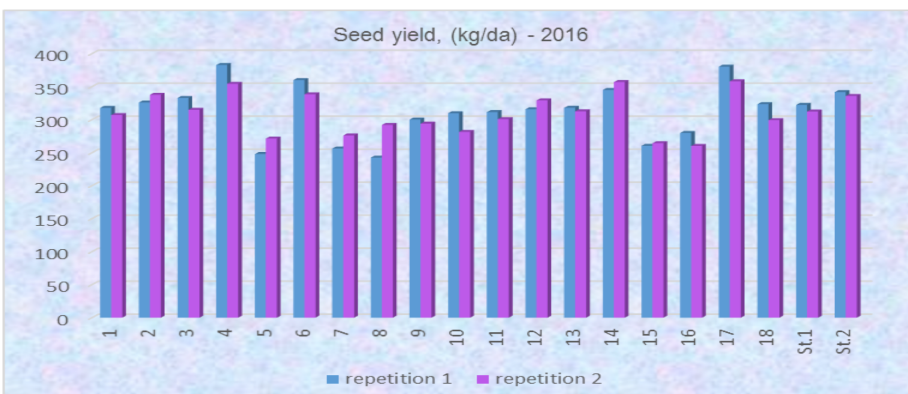

Fig. 2. The seed yield in 2016 of 18 experimental hybrids and standards

If occurring in that period, the drought causes substantial reduction of yield and yield components. That's why the seed yield of experimental hybrids and the mean standards Adagio-CX and LG 5661 CL were significantly lower in 2016 than in 2017 (fig.2, 3).

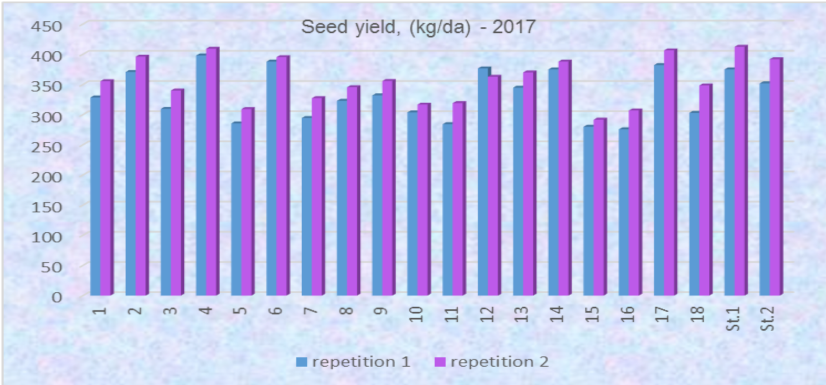

Fig. 3. The seed yield in 2017 of 18 experimental hybrids and standards

The mean standard for seed yield of replications in 2016 was 337,2 kg/da. Just three of the tested experimental hybrids exceeded it with 3,6% to 6,4%. The best results showed the hybrids 864 A x 43 R and 864 A x 55 R and 802 A x 43 R. Their seed yield was respectively 358,7 kg/da, 349,3 kg/da and 354,3 kg/ha. They also exceeded the mean standard of oil yield with 1,8% - 3,8% respectively. The mean standard for seed yield of replications in 2016 was 136,7 kg/da. The lower seed yield was due to the lack of water supply in the soil and rainfall during the vegetation period of plants. The exceeding of seed oil content was 3,6% to 12,5% for some of the studied hybrids. The exceeding regarding oil yield varied from 4,1% to 9,2%. The highest seeds oil content was determined for hybrid 1111A x 55 R – 47,5%, followed by 864 A x 43 R – 46,5%. The mean standard for seed yield of replications in 2017 was 382,9 kg/da. Four of the tested experimental hybrids exceeded it with 1,2% to 6,7%. The best results showed the hybrids 864 A x 43 R (408,7 kg/da), 864 A x 18 R (398,9 kg/da), 802 A x 43 R (391,7 kg/da) and 1111 A x 14 R (387,5 kg/da). They also exceeded the mean standard of oil yield with 2,4% - 5,8% respectively (fig.4).

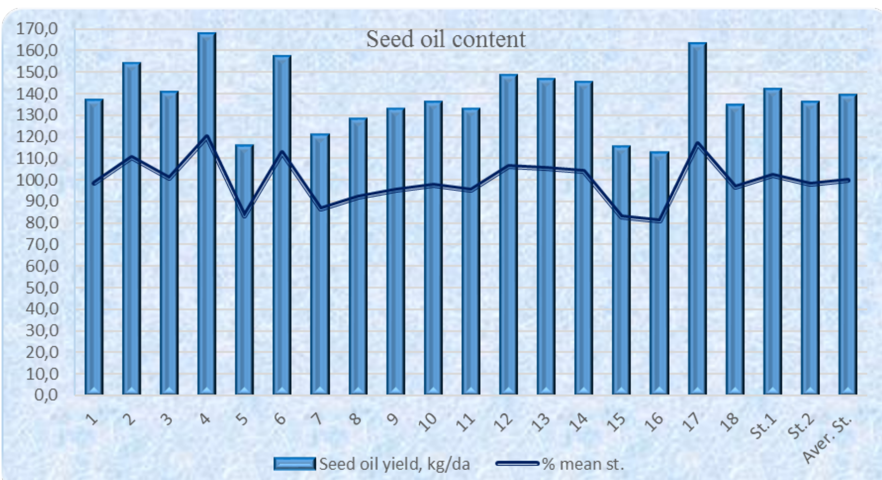

Fig. 4. Average seed oil content of 18 experimental hybrids and standards (2016-2017).

Two-way analysis of variance was applied for two independent variables with aim to determine three sets of hypothesis.

Table 1. Dispersion analysis of variance for some characters of tested hybrids, connected to seed yield.

Characters	MSh	MSy	MS yxh	MS error	LSD 5%	LSD 1%	LSD 0,1%
Seed oil content	7,15**	69,1***	1,7	0,94	0,62	0,73	1,01
Oil yield	1193,2***	4213,1***	81,4	58,4	5,16	6,71	8,17

Seed yield	3743,1**	9230,7***	328	410	12,7	15,2	21,5
Plant height	4112,3	2235***	1312,9***	33,5***	3,2	4,4	6,1
Head diameter	137,7***	31,1**	18,1*	2,1	1,1	1,4	1,4

** - statistical significant by p=0.01 , *** - statistical significant by p=0.001

The null hypotheses for each of the sets are the follow: 1.The population means of the first factor are equal. 2. The population means of the second factor are equal. 3. There is no interaction between the two factors.

The idea is that there are two variables, factors, which affect the dependent variable. Each factor will have two or more levels within it, and the degrees of freedom for each factor is one less than the number of levels. The summarized results were demonstrated on table 1. There is an F-test for each of the hypotheses, and the F-test is the mean square for each main effect and the interaction effect divided by the within variance. The numerator degrees of freedom come from each effect, and the denominator degrees of freedom is the degrees of freedom for the within variance in each case. The critical value of F is a function of the degrees of freedom of the numerator and the denominator and the significance level. If $F > F_{crit}$, the null hypothesis is rejected. An expressive interaction existed between year and hybrid in their influence on the seed yield and $F_{-criteria}$, pointed that the hypothesis for significance of compound effects was rejected. As the compound effects of these two factors were significant, it could be accepted that the influence of factors *year* and *hybrid* on the seed yield was significant.

The reaction of experimental hybrids to the pathogens *Plasmopara helianthi, Phomopsis helianthi, Phoma macdonaldii* and the parasite broomrape (*Orobanche cumana*) was studied with aim to establish the resistant ones (Tables 2-3). The hybrid combinations 802 A x 14 R, 802 A x 43 R, 864 A x 43 R, 864 A x 51 R, 864 A x 55 R were resistant (100%) to downy mildew and the parasite broomrape. They were characterized with resistant type of reaction to the pathogens caused grey and black spots on sunflower and with up to 49% oil content in seeds. Their vegetation period was 111-118 days. Certain resistance to these two pathogens was established for the other hybrids, obtained with participation of resistant to these pathogens restorer lines. These hybrids could be successfully included in the sunflower breeding programs for developing new resistant lines.

Table 2. Phytopathological evaluation of F_1 hybrids for resistance to *Pl. helianthi* and *Orobanche cumana.*

Resistance, %	Experimental Hybrids	
Resistance 100 % to *Pl. helianthi* Novot. and 76-99% to *Orobanche cumana* Wallr.	802 A x 14 R 802 A x 43 R 864 A x 43 R	864 A x 51 R 864 A x 18 R 564A x 48R
Resistance 76-99% to *Pl. helianthi Novot*. and *Orobanche cumana* Wallr.	864 A x 18 R 864 A x 48 R 864 A x 56 R 802 A x 48 R	802 A x 14 R 802 A x 51 R 1111 A x 14 R 1111 A x 53 R

Table 3. Phytopathological evaluation of F_1 hybrids for resistance to *Phomopsis helianthi Munt.-Cvet. et all.* and *Phoma macdonaldii Boerema.*

Type of reaction	Experimental Hybrids	
Resistant to *Phomopsis helianthi* and *Phoma macdonaldii*	1111 A x 14 R 1111 A x 18 R	802 A x 43 R 1111 A x 51 R
Mid. resistant to *Phomopsis helianthi* and *Phoma macdonaldii*	864 A x 43 R 864 A x 48 R 864 A x 55 R	864 A x 50 R 802 A x 14 R

CONCLUSIONS

The results showed that the experimental hybrids were distinguished on their ecological plasticity. The lowest susceptibility to different climatic conditions were established for the hybrids 864A x 18 R, 864 A x 43 R, 1111A x 14 R and 802 A x 43 R. These hybrids were characterized with resistance to downy mildew and broomrape, highest seed oil content and oil yield respectively. They were submitted to continue the official variety testing. All other hybrids will be included in third-year testing for choosing those with high resistance to biotic and abiotic stress factors, high oil content in the seed.

REFERENCES

Al-Khatib, K., J.R. Baumgartner, D.E. Peterson, R.S. Currie. 1998. Imazethapyr resistance in common sunflower (*Helianthus annuus*). Weed Science, 46: 403–407.

Al-Khatib, K., J.F. Miller. 2000. Registration of four genetic stocks of sunflower resistant to imidazolinone herbicides. Crop Science, 40: 869–870.

Barov, V. J. Shanin. 1965. Methodology of field experiments. Sofia.

Bruniard, J.M., J.F. Miller. 2001. Inheritance of imidazolinone herbicide resistance in sunflower. Helia 24: 11–16.

Encheva V. and I. Kiryakov. 2002. Method for evaluation of sunflower resistance for *Diaporthe (Phomopsis*) *helianthi* Munt. Cnet. et al. Bulgarian Journal of Agricultural Science 8:219-222.

Evci, G., V. Pekcan, M. I. Yılmaz, Y. Kaya. 2012. The Resistance breeding to IMI and SU herbicides in sunflower. Plant Science, 49, 6-11.

Fayralla ES and Maric A, 1981. Prilog proucavanju biologiye I epidemiologije *Phoma macdonaldii* Boerema pouzrokovaca crne pegavosti suncokreta. Zaštita bilja, 32, 13-27.

Kaya, Y. 2014. Sunflower. A. Pratap. (Ed) Alien Gene Transfer in Crop Plants, Vol. 2. Springer Press. 281-315.

Kaya, Y., G. Evci, V. Pekcan, I. M. Yilmaz. 2013. Clearfield Technology in Sunflower and Developing Herbicide Resistance Sunflower Hybrids. Soil-Water Journal. 2(2): 1713-1720.

Rushkovski, C. B. 1957. Metodi issledovanii pri selekcii maslichnih rastenii na soderjanie masla i evo kachestva. -M., Pishtepromizdat. 119 p. (In Russian)

Sala, C. A., M. Bulos, A. M. Echarte, S. Whitt, G. Budziszewski, W. Howie, B. Singh, B.

Sala, C.A., M. Bulos, A. M. Echarte. 2008b. Genetic analysis of an induced mutation conferring imidazolinone resistance in sunflower. Crop Science 48:1817-1822.

Sala, C.A., M. Bulos, E. Altieri, M. L. Ramos. 2012. IMISUN tolerance is the result of theinteraction between target and non-target tolerance mechanisms. In: Proc. 18th Sunflower Conf., Mar del Plata-Balcarce, Argentina, pp. 551-556.

Skoric, D. 2012. Sunflower breeding. In: Skoric, D. (editor), Sunflower genetics and breeding. Serbian Academy of Sciences and Arts, Novi Sad, Serbia, pp. 165-354.

Tan, S., R. R. Evans, M. L. Dahmer, B. K. Singh, D. L. Shaner. 2005. Imidazolinone-tolerant crops: history, currentstatusandfuture. Pest Management Sci.;61(3):246-57.

Vear F.and D. Tourvieille.1987. Test de resistance au Mildiou chez le tournesol.- CETIOM. Information techniques, vol.98, p.p.19-20.

Weston. 2008a. Development of CLHA-Plus: a novel herbicide tolerance trait in sunflower conferring superior imidazolinone tolerance and ease of breeding. In: Proc. XVII Int. Sunfl. Conf., Cordoba, Spain, pp. 489–494.