

## *Effect of Main Climatic Parameters on Some Morphological and Qualitative Characteristics of Doubled Haploid Sunflower Lines*

*Miglena A. Drumeva<sup>\*</sup>, Peter S. Yankov*

Technical University of Varna, Faculty of Manufacturing Engineering and Technologies,  
Department of Plant Production, 1 Studentska str., Varna 9010, BULGARIA

<sup>\*</sup>Corresponding author: m\_drumeva@abv.bg

**Abstract.** The dynamics of changing climatic factors and the study of the impact they have on cultivated crops is an area that needs to be carefully and thoroughly researched in order to adequately address the future challenges of human nutrition. The present study concerned the influence of major environmental factors on some morphological and economic characteristics of sunflower (*Helianthus annuus* L.), which is a main oil crop in Bulgaria. Development and selection of parental lines with stabilized morphological and economic parameters is a main point of the heterosis breeding in sunflower. This study was carried out during 2009-2011 at Dobrudzha Agricultural Institute - General Toshevo. The three years of the investigation differed by the sum of vegetative rainfalls. The investigation involved 10 doubled haploid fertility restorer lines. The analysis of the results showed that the year conditions and the genotype of the investigated lines had a significant effect ( $p=0.001$ ) on the studied morphological traits and on the oil content in seeds. The combined influence of these two factors was not significant on plant height and oil content. The head diameter was influenced to a much higher degree by the year conditions than the plant height. The oil content in seed was the trait with lowest variation during the period of investigation in comparison to the two morphological traits. The highest values of the morphological traits and the content of oil in seed were determined in the warm and humid year 2010.

**Key words:** sunflower, doubled haploid lines, morphological traits, oil content in seed.

### **Introduction**

Sunflower (*Helianthus annuus* L.) is one of the main oil seed crops both in Bulgaria and worldwide. The leading trend nowadays is heterosis breeding of sunflower, its major reference points being high plasticity and adaptability of the sunflower plant ensuring sustainable yields and seed quality under changing climatic factors. To produce a good sunflower hybrid, it is necessary to develop suitable parental components,

which, when crossed, would give hybrid varieties with high productivity and very good resistance to biotic and abiotic factors (Cheres et al., 2000; Škorić, 2009). In the last 18 years, together with the conventional breeding methods, a number of biotechnology methods for developing sunflower lines with valuable economic properties are being extensively utilized for the needs of heterosis breeding. By using gamma-induced parthenogenesis in combination with conventional breeding

methods of evaluation and selection, the developing time for new sunflower lines is shortened considerably (Todorova et al., 1997a; Todorova et al., 1997b; Drumeva et al., 2005; Drumeva, 2012b). The qualitative traits of the lines are dependent on the gene plasma of the initial hybrids these lines were derived from.

The production of hybrid sunflower seeds is preceded by the seed production of their parental lines, which should be of very high genetic purity. This process is rather specific and related to considerable financial investment; therefore knowing the potential of the individual lines is crucial (Georgiev et al., 2004).

Many factors influence the variation of the sunflower morphological indices, productivity and quality. The potential of the genotype plays a decisive role for the quantification of the separate traits, but its expression depends also on the effect of the abiotic factors of the environment, the main component of which are the climatic factors.

The most significant from the climate factors are the quantity of rainfall and the temperature over the vegetation (Hunyadi et al., 2007). Their interaction underlie the formation of quantity and quality of the yield-forming elements in sunflower (Johnston et al., 2002). Increasing of yield is influenced not only by total rainfalls but also by their uniform distribution during the vegetation period, i.e. during the growth stages, when the crop uses them effectively. The sunflower requires higher precipitation during the formation of the assimilatory apparatus with maximum in growth stage of flowering and formation of heads (Černý et al., 2014). The increasingly sharper variations of the climatic conditions put to test the grown crops and although there are different mechanisms for their mitigation, the selection of parental forms with complementing characteristics remains decisive (Škorić, 2009). Due to its ability to grow in different agroecological

conditions and its moderate drought tolerance, sunflower may become the oil crop of preference in the future, especially in the light of global environmental changes (Miladinović et al., 2019).

Determining the range of variation and ecological plasticity of the separate traits allows their more precise assessment and successful performance of selection during the breeding process (Petakov, 1994).

The aim of this investigation was to study the effect of the main climatic parameters - rainfalls and temperature on the value of some morphological and qualitative characteristics of doubled haploid fertility restorer lines in sunflower.

### **Material and Methods**

The experimental work encompassed the period 2009-2011 and was carried out in the experimental fields of Dobrudzha Agricultural Institute - General Toshevo. The investigation involved 10 doubled haploid lines restorers of fertility, which were obtained from different hybrid combinations: Al\_r1 (Albena x 147 R x 600 Gy), Hel\_r2 (Heliasol x 147 R x 600 Gy), Di\_r3 (Diabolo x 147 R x 600 Gy), Br\_r4 (Brio x 147 R x 600 Gy), Ar\_r5 (Arena x 147 R x 600 Gy), Op\_r6 (Opera x 147 R x 600 Gy), Gy), Br\_r4 (Brio x 147 R x 600 Gy), Ar\_r5 (Arena x 147 R x 600 Gy), Op\_r6 (Opera x 147 R x 600 Gy), Br\_r4 (Brio x 147 R x 600 Gy), Ar\_r5 (Arena x 147 R x 600 Gy), Op\_r6 (Opera x 147 R x 600 Gy).

Line 147 R, the father line of hybrid Albena, was used a control. The lines, included in this investigation, were obtained by the methodology of Todorova et al. (1997) through pollination of the initial hybrids with pollen preliminary irradiated with high gamma-ray doses in combination with embryo culture. The pollen was irradiated with dose 600 Gy using Cs 137 as a source of ionizing radiation. The isolation and cultivation of

the obtained embryos was carried out according to Azpiroz et al. (1988).

The experiment was conducted in randomized block design, in three replications, the size of the experimental plot being 20 m<sup>2</sup>.

Biometrical measurements were done on 10 plants from each line for the parameters plant height and head diameter. Plant height was measured from the surface of the soil to the place of head attachment to the stem. The reading was carried out at stage physiological maturity (15-20 days prior to harvesting). At the same time, the head diameter was also determined by doing two perpendicular measurements.

Oil content in seed was determined through nuclear magnetic resonance (Gronlund & Zimmerman, 1975).

Data were processed statistically with the help of the software package SPSS 16.0. (IBM Corp, 2007).

### Results and Discussion

The three years of investigation differed by the sum of vegetative rainfalls; precipitation was highest in 2010 - 369.1 mm (Fig. 1). In 2009 and 2011 the rainfalls during April - September were with 16.5% and 5.9%, respectively, less than the norm.

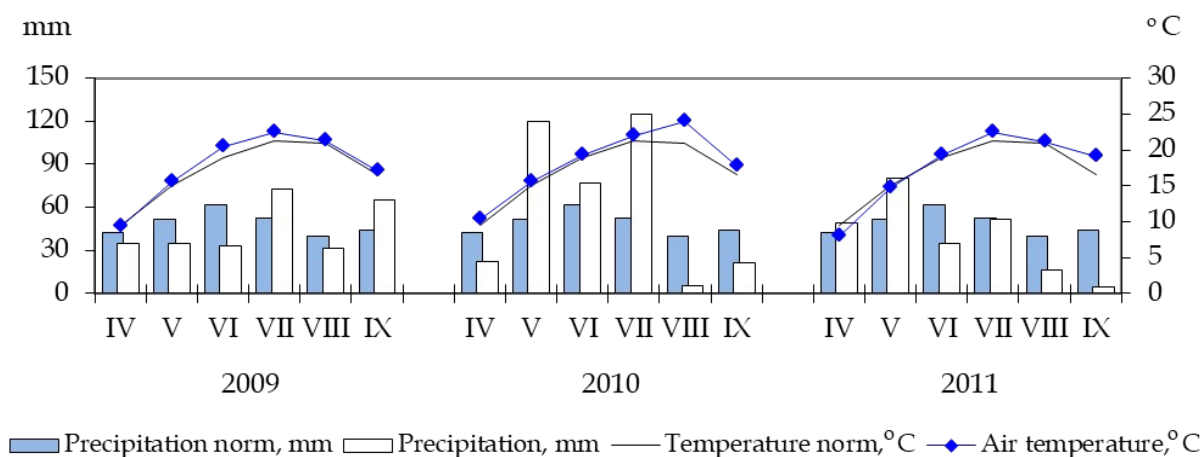
The values of the mean monthly air temperatures are significantly important for the development of the plants and the formation of the yield components in sunflower. Among the three years of investigation, year 2010 was with the highest air temperature during the vegetative growth of plants - 18.2 °C. Values close to the average for the long term period were registered during the other two years, year 2009 being a little warmer than 2011.

In all three years of the investigation, the air temperature was within the range

recommendable for sunflower, but the better moisture reserves in soil during 2010 were a prerequisite for the better development of the crop. During the vegetative growth season of sunflower, the effect of precipitation exceeded the effect of temperatures, but a synchronization between the metabolic requirements of the plant and the climatic factors is needed for the optimal occurrence of the biological processes in the plant cells (Kudrna, 1985).

The analysis of the results showed that the year conditions and the genotype of the studied doubled haploid lines influenced significantly ( $p=0.001$ ) the investigated morphological traits, as well as the percentage of oil in seed (Table 1). The combined effect of these two factors was not significant on plant height and oil content.

Under the conditions of this investigation, the value of the traits plant height and oil content in seed was determined primarily by the genotype, the relative weight of this factor being 62.66% and 69.97%, while the determining significance of head diameter was that of the climatic year conditions (61.52%). The gene effect of head diameter is often minor compared to other agronomic traits, because it depends on the environmental condition and the vegetation period (Marinković & Škorić, 1990; Miller & Fick, 1997; Škorić, 2009). The combined effect of the investigated traits was with low relative weight, varying from 1.46% for plant height to 2.91% for oil content in seed, and reaching up to 6.29% for head diameter. The probable reason for the absence of statistical significance of the mutual effect of the two factors is the good plasticity of the sunflower plant, which demonstrated its adaptability according to the changes in the vegetative growth conditions over the years of study.



**Fig. 1.** Monthly values of rainfalls and air temperature from April till September during 2009-2011.

**Table 1.** Analysis of variance of year (A)  $\times$  line (B) in sunflower for plant height, head diameter and seed oil content.

Indices	Source of variation	df	F	Sig
Plant height (cm)	Factor A. Years	2	422.354	.000
	Factor B. Lines	10	147.542	.000
	A $\times$ B	20	1.717	.053
Head diameter (mm)	Factor A. Years	2	593.385	.000
	Factor B. Lines	10	62.107	.000
	A $\times$ B	20	6.066	.000
Oil content (%)	Factor A. Years	2	132.912	.000
	Factor B. Lines	10	68.577	.000
	A $\times$ B	20	1.427	.142

Plant height is a varietal trait related to productivity, which is also influenced by the meteorological conditions, primarily by the vegetative rainfalls. The investigated lines differed by plant height; averaged for the three years of investigation, the lowest values were read in line Al\_r1 - 126 cm, and the highest - in line Ba\_r7 - 167 cm (Table 2). The differences in the value of this trait between the separate years of study were significant at various levels (Fig. 2).

Among the lines, included in the experiment, line Br\_r4 was with the lowest

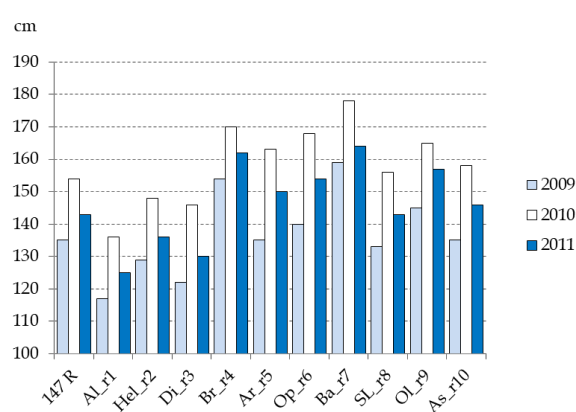
variation coefficient, followed by lines Ba\_r7 and Ol\_r9, and line Ar\_r5 was with the highest coefficient of variation. The different lines had different potential for overcoming of the unfavorable environmental factors and their response can be considered a result from the interaction of the genotype with the factors of the environment. In this investigation, the lines were grown under equal conditions and it can be assumed that the more narrow variation of the investigated parameters is due to the better adaptability of a given line to the ecological factor.

**Table 2.** Mean values of main morphological and qualitative traits of the lines during the period of study.

DHR lines	Plant height (cm)	CV (%)	Head diameter (cm)	CV (%)	Oil content in seed (%)	CV (%)
	$\bar{x} \pm SD$		$\bar{x} \pm SD$		$\bar{x} \pm SD$	
147 R	144.0 ± 9.54	6.62	15.1 ± 2.15	14.24	37.5 ± 1.48	3.96
Al_r1	126.0 ± 9.54	7.57	13.2 ± 2.36	17.91	36.4 ± 0.92	2.52
Hel_r2	137.7 ± 9.61	6.98	15.4 ± 1.72	11.17	34.6 ± 1.15	3.33
Di_r3	132.7 ± 12.22	9.21	13.3 ± 2.25	16.93	35.3 ± 1.10	3.12
Br_r4	162.0 ± 8.00	4.94	16.4 ± 1.57	9.58	38.0 ± 1.51	3.97
Ar_r5	149.3 ± 14.01	9.38	13.8 ± 2.65	19.28	36.5 ± 1.55	4.25
Op_r6	154.0 ± 14.00	9.09	14.3 ± 3.10	21.74	37.5 ± 1.66	4.41
Ba_r7	167.0 ± 9.85	5.90	16.7 ± 1.31	7.80	35.8 ± 1.01	2.81
SL_r8	144.0 ± 11.53	8.01	15.7 ± 1.61	10.25	38.6 ± 1.36	3.51
Ol_r9	155.7 ± 10.07	6.47	15.9 ± 1.56	9.85	33.3 ± 0.71	2.13
As_r10	146.3 ± 11.50	7.86	15.0 ± 1.40	9.36	34.4 ± 1.20	3.49

The highest mean values according to this index were read in 2010 against the background of 348.1 mm precipitation during the vegetative growth of plants (April – August) and mean air temperature of 18.2 °C (Fig. 2). Under these conditions, the variation in plant height of the investigated fertility restorer lines was within the range 136-178 cm. The lowest mean values (117-159 cm) were registered in 2009 at 206.4 mm and 17.8 °C mean air temperature. The difference in the rainfalls between the experimental years was 141.7 mm, and this difference reduced the marginal values of the investigated trait with 19 cm during the dry year 2009. The value of the trait was within 125-164 cm in 2011 at 232.8 mm of rainfalls during the vegetative growth of plants and 17.1 °C mean air temperature.

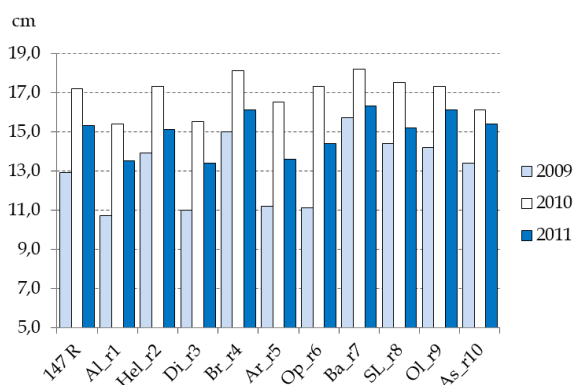
Averaged for the three years, the fertility restorer lines demonstrated values of head diameter within the range from 13.2 to 16.7 cm (Table 2), which corresponded to values of head diameter in branched fertility restorers determined by other authors as well (Georgiev et al., 2004).



**Fig. 2.** Mean values of trait plant height (cm) of the lines during different years from the investigation.  $p_{0.05}=7.978$ ;  $p_{0.01}=9.939$ ;  $p_{0.001}=14.237$

The value of this trait was highest in 2010, and lowest – in 2009, with differences significant at  $p=0.001$  (Fig. 3). The lowest value of this parameter in 2010 was 15.4 cm in line Al\_r1, and the highest was the value in 2009 – 15.7 cm in line Ba\_r7. The minimal values of this parameter overlapped in 2010, with an insignificant difference of 0.3 cm with the maximal value of the trait in 2009.

These results showed that the head diameter was influenced to a much greater degree by the year conditions in comparison to plant height, which was confirmed also by the relatively higher coefficient of variation according to this trait in the greater part of the investigated lines (Table 2). Averaged for the three experimental years, the variation according to this trait among the investigated lines was within the range 7.80% - 21.74%, delineated by lines Ba\_r7 and Op\_r6. Line Ba\_r7 was with the lowest coefficient of variation according to the trait, followed by lines As\_r10 and Br\_r4. Such results, proving the relatively wider variability of the trait in comparison to plant height, depending on the growing conditions, have been reported by other authors, as well (Dušanić et al., 2004; Škorić, et al., 2012). In their investigation, Kaya et al. (2016) found out that plant height was affected less by drought stress, while head diameter of plants decreased with up to 50% under drought stress conditions.



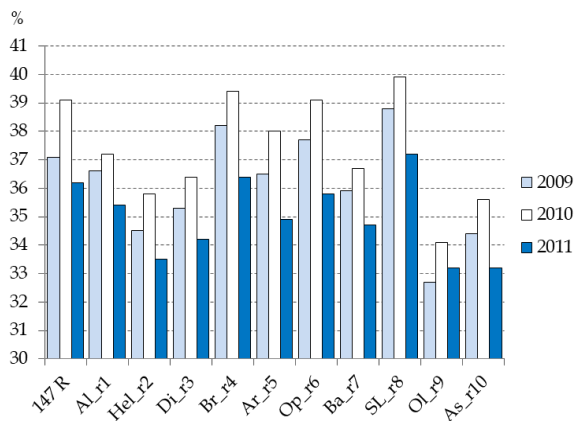
**Fig. 3.** Mean values of head diameter (cm) of the investigated lines in different years of investigation.  $p_{0.05}=1.778$ ;  $p_{0.01}=2.290$ ;  $p_{0.001}=3.412$

Oil content in seed is a parameter of primary technical importance. The polygenic nature of the trait, in which dominance effects are observed, allows obtaining hybrids with high oil content even when only one of the lines is with high oil percentage. Since sunflower is an oil seed crop, this parameter is with high breeding value and a large part of the breeding

programs related to developing oil seed sunflower hybrids, have to do with increasing the values of this parameter in the parental lines of such hybrids (Hladni et al., 2006; Hladni et al., 2008; Drumeva, 2012a). Therefore, negative deviations in its values are highly undesirable from a breeding point of view. Oil content in the seed of the investigated lines varied from 33.3% in OL\_r9 to 38.6% in SL\_r8, averaged for the three years of investigation (Table 2). The most favorable conditions for accumulation of oil in seed were in the warm and humid year 2010, when a maximum of 39.9% oil was registered in the seeds of line SL\_r8 (Fig. 4). The seed oil content of this line in 2011 was 37.2%, and in 2008 - 38.8%. The difference in the oil content determined in these two years was not significant. Lines Br\_r4 and Op\_r6 also possessed high oil content.

It is worth mentioning that the highest absolute percent of oil in the lines was registered in 2009, which was drier than 2011. The differences in the precipitation during the vegetative growth between these two years was 26 mm in favor of 2011 for the entire vegetation period, but the amount of rainfalls in July of the drier year 2009 significantly exceeded the amount of rainfalls during the same month of year 2011 (Fig. 1). This is the month, in which the moisture reserves in soil available to the plants are especially important because of the beginning of the grain filling stage. According to a number of authors (Černý et al., 2014; Škorić, 2009; Škorić et al., 2012; Stanojevic et al., 1992), the oil content in seed is determined by the genotype and the agro ecological conditions, the effect of which is especially evident at the seed filling stage. The insufficient precipitation has a strong effect on the metabolic processes of the sunflower plant, especially at the stage of increased demand for physiological moisture resulting in lower oil content accumulated in the seed. According to Škorić (2009), drought at this stage causes seed oil percentage to decrease by 7-8%. This was probably the reason for the averagely higher

oil content of the lines read during the experimental year 2009, when the amount of rainfalls in July was 72.4 mm, and in 2011 – 51.8 mm. Regardless of the differences determined in the absolute values of the lines according to this index, the variation of the mean values was rather low, indicating considerable stability of the parameter. Oil percent in seed was the trait with lowest variation during the period of study in comparison to the two morphological traits (Table 2).



**Fig. 4.** Mean values of oil content in seed (%) of the investigated lines over years.  $p_{0.05}=1.917$ ;  $p_{0.01}=2.521$ ;  $p_{0.001}=3.845$

### Conclusions

The year conditions and the genotype of the investigated doubled haploid lines had a significant effect on the studied morphological traits and on the oil percentage in seed. The combined effect of these two factors was not significant on plant height and oil in seed. Under the conditions of this investigation, the values of the traits plant height and oil content in seed were determined mainly by the genotype, the relative weight of this factor being respectively 62.66% and 69.97%, while the climatic year conditions had decisive impact on head diameter (61.52%).

The highest values with regard to the morphological traits and oil percent in seed were read during the warm and humid year 2010.

Under the conditions of this investigation, line Br\_r4 was with the best adaptability to the conditions of the environment; in it, the variation of the mean values of all three traits was relatively low for the three-year period of the investigation. The line also had high oil percentage, and together with lines SL\_r8 and Op\_r6, also with high oil percent, can be used in breeding programs for developing oil seed sunflower hybrids.

### References

- Azpiroz, H., Vincourt, P., Serieys, H., & Gallais, A. (1988). La culture in vitro des embryons immatures dans l'accélération du cycle de sélection des lignées de tournesol et ses effets morphovegetatifs. *Helia*, 10, 35-38.
- Černý, I., Veverková, A., Kovár, M., Pačuta, V., & Molnárová, J. (2014). Influence of temperature and moisture conditions of locality on the yield formation of sunflower (*Helianthus annuus* L.). *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 59(6), 99-104. doi: [10.11118/actaun201159060099](https://doi.org/10.11118/actaun201159060099).
- Cheres, M., Miller, J., Crane, J., & Knapp, S. (2000). Genetic distance as a predictor of heterosis and hybrid performance within and between heterotic groups in sunflower. *Theoretical and Applied Genetics*, 100, 889-894. doi: [10.1007/s001220051366](https://doi.org/10.1007/s001220051366).
- Dušanić, N., Miklič, V., Joksimović, J., & Atagić, J. (2004). Path coefficient analysis of some yield components of sunflower. In *Proceedings of the 16th International Sunflower Conference*, (pp. 531-537), Fargo II.
- Drumeva, M. (2012a). Development and testing of experimental sunflower hybrids obtained by using doubled haploid lines. *Agricultural Science and Technology*, 4(3), 196-200.
- Drumeva, M. (2012b). Developing sunflower fertility restorer lines from commercial hybrids by using in vitro technique.

- Agricultural Science and Technology*, 4(4), 361-364.
- Drumeva, M., Berville, A., Ivanov, P., Nenova, N., & Encheva, J. (2005). Molecular investigations on the doubled haploid origin of sunflower lines (*Helianthus annuus* L.) developed through gamma-induced parthenogenesis. *Biotechnol. & Biotechnological Equipment*, 19(3), 46-50. doi: [10.1080/13102818.2005.10817226](https://doi.org/10.1080/13102818.2005.10817226).
- Georgiev, G., Encheva, V., Nenova, N., Peevska, P., Encheva, Y., Valkova, D., Georgiev, G., & Penchev, E. (2014). Characterization of the yield components of sunflower lines under the conditions of North-East Bulgaria. *Field Crops Studies*, 9(2), 249-258. (In Bulgarian).
- Gronlund, M., & Zimmerman, D. (1975). *North Dakota Acad. Sci. Proceedings*, 27(2).
- Hladni, N., Jocić, S., Miklič, V., Mijić, A., Saftić A., Panković, D., & Kraljević-Balalić, M. (2008). Direct and indirect effects of morphophysiological traits on oil yield of sunflower (*Helianthus annuus* L.). In *Proceedings of the International Conference on Conventional and Molecular Breeding of Field and Vegetable Crops*, (pp. 491-494), November 24-27, 2008, Novi Sad, Serbia.
- Hladni, N., Škorić, D., Kraljević-Balalić, M., Sakač, Z., & Jovanović, D. (2006). Combining ability for oil content and its correlations with other yield components in sunflower (*Helianthus annuus* L.). *Helia* 29(44), 101-110. doi: [10.2298/hel0644101h](https://doi.org/10.2298/hel0644101h).
- Hunyadi Borbély, É., Csajbók, J., & Lesznyák, M. (2007). Relations between the yield of sunflower and the characteristics of the cropyear. *Cereal Research Communications*, 35(2), 285-288.
- IBM Corp. (2007). SPSS software ver. 16.0. Retrieved from [www.spss.com](http://www.spss.com).
- Johnston, A., Tanaka, D., Miller, P., Brandt, S., Nielsen, D., Lafond, G., & Riveland, N. (2002). Oilseed Crops for Semiarid Cropping Systems in the Northern Great Plains. *Agronomy Journal*, 94, 231-240. doi: [10.2134/agronj2002.2310](https://doi.org/10.2134/agronj2002.2310).
- Kaya, Y., Pekcan, V., & Cicek, N. (2016). Effects of drought on morphological traits of some sunflower lines. *Ekin Journal of Crop Breeding and Genetics*, 2(2), 54-68.
- Kudrna, K. (1985). *Zemědělské soustavy*. Praha: SZN.
- Marinković, R., & Škorić, D. (1990). Nasleđivanje prečnika glave i broja cvetova po glavi u ukrštanjima raznih inbred linija suncokreta (*Helianthus annuus* L.). *Uljarstvo*, 27(1-2), 22-27.
- Miladinović, D., Hladni, N., Radanović, A., Jocić, S., & Cvejić, S. (2019). Sunflower and Climate Change: Possibilities of Adaptation Through Breeding and Genomic Selection. *Genomic Designing of Climate-Smart Oilseed Crops*, 173-238. doi: [10.1007/978-3-319-93536-2\\_4](https://doi.org/10.1007/978-3-319-93536-2_4).
- Miller, J., & Fick, G. (1997). The genetics of sunflower. *Sunflower technology and production*, 35, 441-495. doi: [10.2134/agronmonogr35.c9](https://doi.org/10.2134/agronmonogr35.c9).
- Petakov, D. (1994). Correlation and heritability of some quantitative characters in sunflower diallel crosses. In *Proceedings of symposium on breeding of oil and protein crops*, (pp. 162-164), Albena, Bulgaria.
- Škorić, D. (2009). Sunflower breeding for resistance to abiotic stresses. *Helia*, 32(50), 1-16. doi: [10.2298/hel0950001s](https://doi.org/10.2298/hel0950001s).
- Škorić, D., Seiler, G., Zhao, L., Chao-Chien, J., Miller, J., & Charlet, L. (2012). *Sunflower genetics and breeding*. International Monography, Serbian Acad. of Sci. and Arts, Branch in Novi Sad.
- Stanojević, D., Nedeljković, S., & Jovanović, D. (1992). Oil and protein concentration in seed of diverse high-protein inbred lines of sunflower. In *Proceedings of 13th International Sunflower Conference Pisa*, (pp. 1263-1268), September 1992, II.



- Todorova, M., Ivanov, P., Shindrova, P., Christov, M., & Ivanova, I. (1997a). Dihaploid plant production of sunflower (*Helianthus annuus* L.) through irradiated pollen-induced parthenogenesis. *Euphytica*, 97, 249-254. doi: [10.1023/A:1002966824988](https://doi.org/10.1023/A:1002966824988).
- Todorova, M., Nenova, N., Ivanov, P., & Christov, M. (1997b). Plant regeneration through anther culture and induced parthenogenesis in Genus *Helianthus*. *Biotechnol. & Biotechnol. Equipment*, 4, 27-30. doi: [10.1080/13102818.1997.10818948](https://doi.org/10.1080/13102818.1997.10818948).

Received: 08.04.2020

Accepted: 17.04.2020