

# NEW SOURCES FOR GENETIC VARIABILITY WITH RESISTANCE AT DROUGHT OBTAINED BY INTERSPECIFIC HIBRIDIZATION BETWEEN CULTIVATED SUNFLOWER AND THE ANNUAL WILD SPECIES *Helianthus argophyllus*

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## Abstract

Taking into account the major climatic changes recorded over the last 10 years, one of the main objectives of sunflower improvement is adaptability to environmental conditions, high productivity in extreme drought and burning conditions. Considering the global warming predicted by experts, the first step was to identify genotypes created at NARDI Fundulea for drought resistance. The new genotypes were obtained by interspecific hybridization of the *Helianthus argophyllus* crop rows. The wild species is recognized as having high drought resistance due to the highly developed root system and foliar pubescence that reduces evapotranspiration. Genotypes identified with increased drought resistance gave a high yield of seeds, a mass of 1000 seeds and high oil content (> 45%) were introduced as new genetic resources interspecific for the improvement of sunflower. The oil content was read using a nuclear resonator. Embryo rescue technique was used to speed up the breeding process. Thus, immature hybrid embryos were inoculated on germination media and the plants were transferred into pots with earth/sand (50/50) then in 10 kg buckets and were grown in the greenhouse, 2 generations/year were obtained.

**Key words:** *Helianthus argophyllus*, embryo rescue, interspecific hybridization, sunflower genotypes, gene introgression of drought resistance.

## INTRODUCTION

In Romania, Vrânceanu (2000) was able to obtain interspecific progenies between *Helianthus annuus* x *Helianthus argophyllus*. Interspecific hybridization is an additional technique to create new sources of genetic variability for the improvement of sunflower (Christov, 2013; Prohens et al., 2018).

According to the information provided by Hussain et al (2017), the evaluation of the descendants obtained by hybridizing the sunflower cultivated *Helianthus annuus* with annual wild species *Helianthus argophyllus* showed improvements in water behavior (water consumption, stomatic behavior), but the characters of productivity, precocity have been diminished.

At the same time, the offspring of the first generation of hybrids were strongly branched, small caps and a long vegetation period (Merrien et al., 1996).

Improving drought resistance is quite complex due to the polygenic control of this character. Many research has been carried out to identify a single character that would serve as a basis for drought resistance selection, but this approach has proved to be unrealistic (Vrânceanu, 2000).

Characters identified as correlated with resistance to water stress are: the average weight of achenes (Andrich et al., 1996), high productivity achieved through a large amount of biomass at maturity, rapid phenological development combined with a long reproduction period and thick stem strains to provide sufficient amounts of carbonated hydrates in the critical seed filling period (Barron, 1991).

Baldini et al. (1993) reported that the annual wild species *Helianthus argophyllus* can be used in drought resistance programs because it absorbs water better during long periods of

drought due to the highly developed root system.

Following the testing and selection under natural climatic and soil conditions on agricultural areas, Saucă (2010a; 2010b), Saucă and Lazăr (2016) reported the existence of a large genetic variability within the newly created interspecific germplasm both for drought resistance and for resistance to various pathogens.

## MATERIALS AND METHODS

In this paper will be presented the results obtained in experimental years 2016-2017 at Fundulea (Calarasi County) and Stupina (Constanta County) where observations were made for: drought resistance, seed production and oil content in new genotypes created.

In the collection from NARDI Fundulea, there are many sunflower genotypes (inbred lines based on cytoplasmic androsterility - A lines, inbred lines maintaining fertility - B lines and fertility restoration lines - C lines, synthetic populations, hybrids) with different degrees of drought resistance.

In order to improve this character without diminishing the seed production and the percentage of seed oil, and in order to support the farmers practicing the classic system, we make interspecific hybridization between 6 fertility maintaining lines (1B-M2, 2B-M4, 3B-M6, 4B-M7, 6B-M11 and M12), and one synthetic population (5B-M10) with the annual wild species *Helianthus argophyllus* in year 2014 followed by generations of backcross and selection in the years 2016 and 2017, obtaining new genetic material (widening the genetic basis of sunflower genotypes) to be used in the process of breeding for various stressors, including drought and heat (Table 1).

Table 1. Name/code of sunflower genotypes

Code of sunflower genotype	Name of sunflower genotype
1B-M2	Polet11B
Bio1	Polet11B x <i>H. argophyllus</i>
2B-M4	O-7493B
Bio3	O-7493B x <i>H. argophyllus</i>
3B-M6	RPC-46B
Bio5	RPC-46B x <i>H. argophyllus</i>

4B-M7	Tard85-2B
Bio7	Tard 85-2B x <i>H. argophyllus</i>
5B-M10	Pop. 79-16B
Bio9	Pop79-16B x <i>H. argophyllus</i>
6B-M11	Tard85-1B
Bio11	Tard85-1B x <i>H. argophyllus</i>
M12	LC-1093B
Bio13	LC-1093B x <i>H. argophyllus</i>

We sowing micro plots with four rows per sunflower genotype (7 m/row), in 3 replications (Figure 1).



Figure 1. Aspects from field with sunflower genotypes tested for resistance to drought, Fundulea 2016

We harvest total number of plants/plot and obtain seed yield per plot and after that we determined seed yield per plant.

$$\text{Seed yield per plant} = \frac{\text{Seed yield per plot}}{\text{Total number of plants per plot}}$$

We determining the oil content with a **Analyzer Spin Track with Nuclear Magnetic Resonance** (Figure 2).

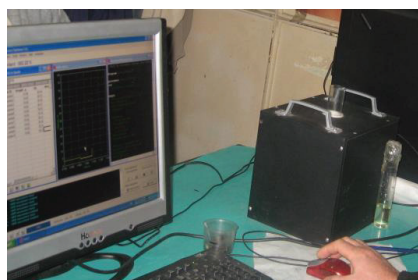


Figure 2. Analyzer Spin Track with Nuclear Magnetic Resonance

## RESULTS AND DISCUSSIONS

During the sunflower growing season (20-25 April - sowing, 20-25 September - harvesting) average temperatures (Figure 3) are in April, at sunflower sowing, the temperatures recorded at both weather stations from Stupina and Fundulea, were higher in 2016 at about 3°C compared to 2017; in July and August, in the phenophase of filling and maturity of the achenes, at Stupina were recorded higher temperatures with 2°C in both experimental years, compared to the station in Fundulea. From Figure 3 it can be noticed that during the experimental period the air temperature at both Fundulea and Stupina in January is below 0°C.

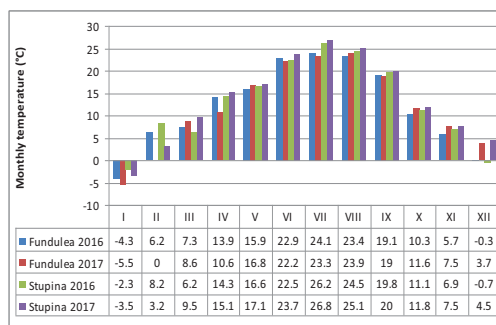


Figure 3. Monthly average temperatures recorded in Fundulea and Stupina in the years 2016-2017

Regarding the amount of rainfall during the calendar years under study, we can conclude that, without exception, the rainfall was significantly higher at Fundulea compared to those recorded at Stupina (Figure 4).

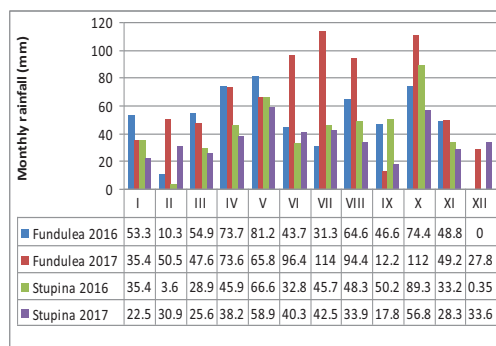


Figure 4. Monthly average rainfall recorded in Fundulea and Stupina in the years 2016-2017

Noteworthy is that under high temperature conditions in April-September, rainfall was significantly low at Stupina in 2017 in the same months. Newly created sunflower genotypes have undergone simultaneous thermal and hydric stress.

From Figure 5 it can be seen that both the fertility maintaining lines and the interspecific hybrids derived from the hybrids produced a higher amount of seeds in the weather conditions at Fundulea. It is noted the Bio 7 genotype that was obtained from interspecific hybridization with the 4B-M7 fertility maintaining line that consistently behaved in both experimental years achieving a higher seed yield/sunflower plant than the 4B-M7 fertility maintaining line both in weather conditions from Fundulea and from Stupina. Also noteworthy are the Bio5 and Bio 11 genotypes that have exceeded seed production over the 3B-M6 and 6B-M11 fertility maintaining lines in both locations in 2016 and 2017.

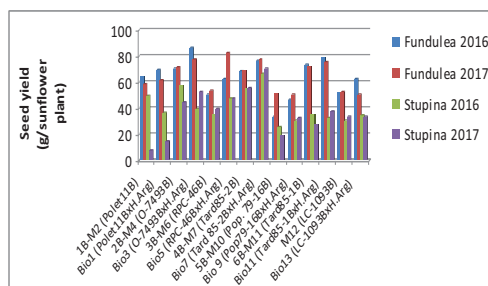


Figure 5. Seed yield (g/sunflower plant), fertility maintaining lines (lines B), synthetic populations and descendants derived from interspecific hybridization between B lines, synthetic populations and the annual wild specie *Helianthus argophyllus* in the years 2016 and 2017 in Fundulea and Stupina

Regarding the oil content, it can be seen from Figure 6 that the Bio 7 and Bio 9 genotypes have an oil content of nearly 50% under the conditions of Fundulea, exceeding the 4B-M7 maintaining fertility line and the synthetic population 5B-M10. And for this character, the Bio 7 genotype exhibits stability and superiority to the 4B-M7 maintaining fertility line over the two years, and the two locations (50%). It is followed by Bio 9 genotypes with an oil content of over 45% and Bio 11 with 44% obtained in the Stupina in 2017.

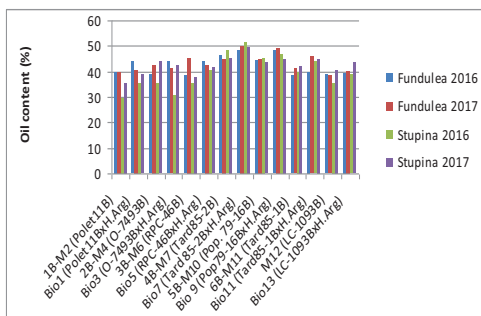


Figure 6. Oil content (%) of newly created genotypes compared to fertile maintenance and synthetic population in the years 2016 and 2017 in Fundulea and Stupina

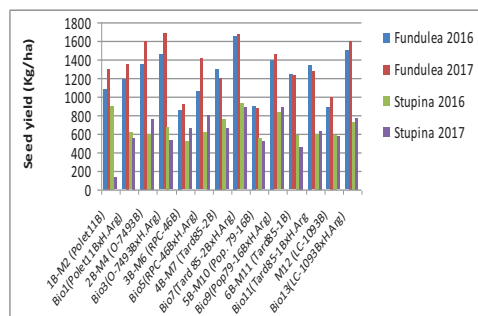


Figure 8. Seed yield (kg/ha) of sunflower genotypes studied in the years 2016 and 2017, in Fundulea and Stupina

1000 weight seed (TWS) is a parameter strongly influenced by the amount of precipitation that fell during of the sowing period (favoring the development of the root system) until at the filling of the seed.

As can be seen in Figure 4, in 2017 in Fundulea, it was a year with many rainfall. This explains the fact that MMB was higher in all genotypes in Fundulea in 2017 and in all cases higher in interspecific descendants compared to the fertile maintenance line and the synthetic population in both locations and experimental years (Figure 7).

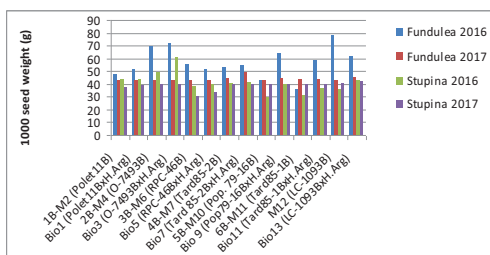


Figure 7. One thousand seed weight of sunflower genotypes, obtained in the years 2016 and 2017 in Fundulea and Stupina

Seed yields (kg/ha) with only a few exceptions were higher in 2017 at Fundulea, a year with rainfall during the whole sunflower growing season and in sufficient quantities in June-July-August.

In the extreme conditions of drought and heat from Stupina we notice the genotype Bio 7, followed by Bio 5 and Bio 13 (Figure 8).

## CONCLUSIONS

Three newly created sunflower genotypes, Bio 7, Bio5 and Bio 11 (Figure 9), were identified as a result of interspecific hybridization with annual wild species *Helianthus argophyllus*, which had a higher seed yield (g/sunflower plant) compared to the genotypes 4B- M7, 3B- M6 and 6B-M11.



Figure 9. Sunflower genotype, Bio 11, in Fundulea, 2017

The genotypes Bio 7, Bio 9 (Figure 10) and Bio 11 have a good oil content and sunflower genotypes Bio7, Bio 9 and Bio 13 have a better 1000 grain weight in both locations, Stupina si

Fundulea and experimental years 2016 and 2017.



Figure 10. Sunflower genotype, Bio 9, in Fundulea, 2017

For seed yield (kg/ha), genotypes Bio7, Bio 9 and Bio 13 (Figure 11) recorded high yields in both locations, Fundulea and Stupina.



Figure 11. Sunflower genotype, Bio 13, in Stupina, 2017

For all the parameters studied, the newly created genotype, Bio 7 (Figure 12), is superior both compared to the fertility maintenance line 4B-M7 and compared to the other genotypes for all studied parameters.



Figure 12. Sunflower genotype, Bio 7, in Fundulea, 2017

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