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Original Research Article

Screening of some sunflower genotypes (*Helianthus annus*) for drought stress using PEG 6000

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Abstract

In 2008 at NARDI Fundulea was initiated a breeding program to improve sunflower for drought resistance by genes introgression from the *Helianthus argophyllus* species into sunflower cultivation lines. Test of new genetic material was represented by hybrid combinations resulting from a selection process that took place over a period of 7 years including all stages of the breeding (hybridization: H. annuus x H. argophyllus, backcross, self pollination and selection). For this study, the testing of genotypes was performed under laboratory conditions to water stress induced by the presence of PEG 6000 in 5 concentrations and 3 replications/variant. Estimating concentrations of optical active substances (the percentage of sucrose equivalent) of the juice extracted by pressing plantlets (with a manual press) with a Refractometer Abbe (Karl Zeiss) was performed. In contrast with plantlets from backcross 7, at higher concentrations of PEG (15% and 20%) many plantlets from maternal lines did not survive until the end of the experience. The 10% PEG 6000 concentration permitted the best analysis of the tested material and it will be maintained in our further studies. For most studied lines were pointed out backcross descendents that exceed their maternal lines and combinations that are bellow the initial levels especially at low stress levels. A number of eight backcross 7 descendents were selected for the final stage of improving for drought resistance, in order to obtain new hybrids.

Key words: Backcross, Helianthus argophyllus, Interspecific hybridization, Self pollination, Water stress, PEG 6000

INTRODUCTION

Improving drought tolerance of sunflower cultivars is a priority for breeding programs because it provides a stable production under climate change already seen in the last twenty years or probable (according to climate model ICTP_RegCM, Boroneanţ et al., 2011) for the rest of the 21st century.

Interspecific hybridization is an additional technique to improve sunflower for new sources of genetic variability (Christov 1996 [2]). With all the difficulties that may arise due to differences in the number of chromosomes (2x, 4x, 6x) and cross incompatibility, interspecific hybridization is considered a way accessible to the incorporation of wild germplasm in to cultivated sunflower, especially for resistance to abiotic stress factors (Vrânceanu 2000; Iouraş and Voinescu, 1984).

In Romania, Vrânceanu et al. (1980) have obtained interspecific progenies *(H. annuus x H. argophyllus)* with drought resistance of sunflower and Vrânceanu and

Iouraş (1988) have selected hybrid genotypes *(H. rigidus x H. annuus)* with good stability (phenotypical and cytological). To maintain valuable genes, the above cited authors have recommended promoting in each backcross generation the useful translocations by selecting individual plants that exhibit the desired phenotype.

At NARDI Fundulea in 2008 was initiated a sunflower breeding program for drought resistance by introgression of genes from *H. argophyllus* species to sunflower inbreed lines (Saucă, 2010).

Along the breeding work with this material, emerged the necessity to have additional *in vitro* information for already field tested genotypes obtained by interspecific hybridization (in the 7th generation of backcross (Saucă et al., 2014). The goal of this paper was to characterize the plantlets growth and the optical activity of the juice extracted from these plantlets after a 14 days exposure to different concentrations of poly (ethylene glycol) (PEG 6000). Selected genotypes will be used to obtain stable lines, greatest as strength to the character and beyond.

The preliminary theoretical difficulty in using optical activity of seedling juice for assessing the capacity of osmo-regulation is at least twofold due to the fact that both the optimal PEG 6000 concentration and time of analysis are unknown. This study was focused on PEG concentration aspect and the dynamics of optical activity (as result of increasing of osmotic active substances and subsequent dilution due to increased water absorption) will be analysed in another work.

Although for technical reasons the optical activity (O.A.) of the seedling juice was expressed like a „sucrose equivalent" percentage (*id est* the sucrose concentration with the same optical activity), the presence of others substances with optic activity in this extract is very probable (White et al., 2009), but most of these compounds are also osmotic active and their concentration will influence the water potential of the cells.

Helianthus argophyllus populations and interspecific hybrids reduced transpiration and improve drought tolerance in cultivated sunflower (Hussain et al., 2016).

The genotype x treatment interactions at different concentration of PEG are identified as most tolerant lines to moisture stress (Shankergoud et al., 2016).

MATERIALS AND METHODS

Genetic material tested was represented by nine sunflower hybrids resulted in seven years by: hybridization *(H. annuus x H. argophyllus)*; backcross, self-pollination and selection.

Please note that for each hybrid combination of selection and hybridization was performed at least four individual plants, so, the results were presented on each plant of hybrid combination which presented resistance to water stress induced by PEG 6000 compared with maternal lines.

The used seeds from the sunflower field improvement from NARDI Fundulea, obtained in the 2013. Five PEG 6000 concentrations were used to wet the paper rolls containing ten seeds for each tested genotype (each in three replications). The used PEG 6000 concentrations were as follows:

V1 = Control (0 % PEG 6000)
V2 = 5.0% PEG 6000
V3 = 10% PEG 6000
V4 = 15% PEG 6000
V5 = 20% PEG 6000

The seeds from the paper rolls were permitted to germinate under laboratory conditions (20 ± 3 ° C) for 14 days. Watering with 30 ml per dish Erlenmeyer of the solutions mentioned above, was applied daily. After 15 days, it has been counted how many of the 10 seeds were germinated, in addition the length of the seedlings was recorded and the juice from plantlets was extracted with a hand press. Measurements of the optical activity of the extracted juice were performed using an Abbe refractometer (Boldor et al., 1983). The results for the genotypes from backcross 7 were displayed graphically against the optical activity for the maternal lines in Excel™ charts and the names of the improved lines (better performances than their maternal lines) were pointed out.

RESULTS AND DICUSSIONS

From our experiments, it was observed that PEG 6000 at concentrations of 5.0% and 10% increase optical activity of solutes in the cell juice (expressed as of sucrose equivalent %). This activity is capped, in the most cases, after overcoming of 15% PEG 6000 concentration.

Although, the significant correlations ($p = 0.05$) were seen between the optical activity of the plantlets from backcross 7 with the optical activity of the maternal lines, it was possible to identify some improved combinations. From figure 1 it can be seen that only one combination (13/5) both plant 1 and plant 2 were responding at the concentration 15% PEG 6000 and the other three combinations (9/1 plant 3; 9/3 plant 1 and 18/1 plant 1) showed optical activity at 10% PEG 6000. These combinations have exceeded the level of resistance of the maternal lines subjected to the same stress induced by PEG 6000.

A better development in the length of seedlings was observed in combinations: 7/3; plant 1 5/1; plant 1; 5/2 plant 2 and 17/2 plant 1 at the concentration of PEG 6000 10% compared with the maternal line. At higher concentrations of plantlets growth were stagnant all other combinations and their maternal lines. However, there is a better resistance genotypes in the seventh gene-ration of retro-crossing compared to the recurrent parent

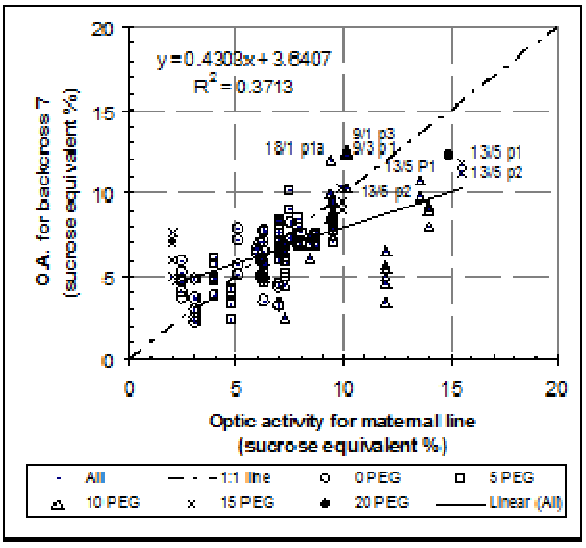

Figure 1. Optic activity for backcross and maternal line for all the testing genotypes

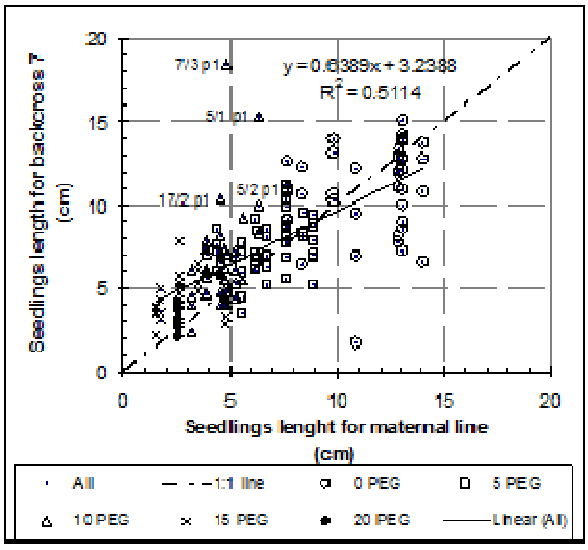

Figure 2. Development of seedlings lenght for all the testing genotypes

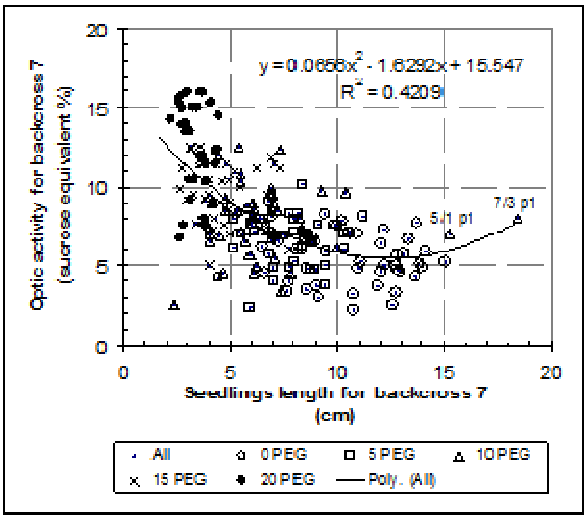

Figure 3. Optic activity and seedlins length for at 7th backcross generation

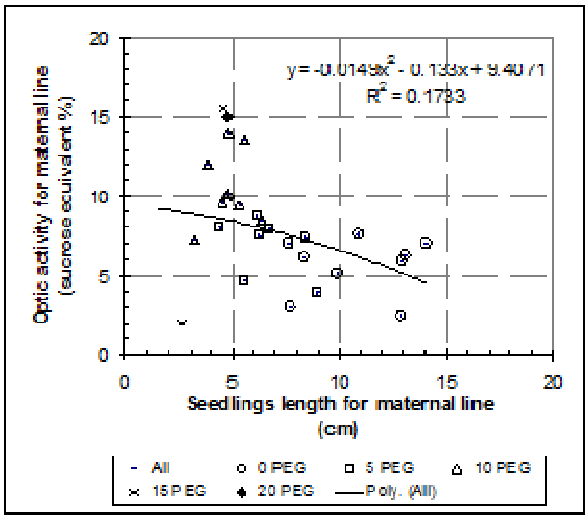

Figure 4. Optic activity and seedlings length for maternal/ paternal lines

genotype and individual screening may help in conjunction with other indicators to maintain that genotype in the breeding process (Figure 2).

At concentration of PEG 20%, most of the seedlings from backcross 7 were able to survive even if the growth of seedlings was clearly reduced (average length was less than 3 cm) but all maternal lines, except line M12 were affected and it was not possible to extract enough juice for O.A. determinations.

At a PEG 15% concentration, for the optical activity of juice extracts from seedling from backcross it was possible a comparison with an increasing number of maternal lines, but the best differentiation was provided by a PEG concentration of 10%. Two backcross 7 genotypes (7/3 p1 and 5/1 p1) had at 10% PEG a growth better than their maternal lines and even better that unstressed plants (0% PEG) (Figure 3,4)

Next its presented individual plant/each combination that were revealed by optical activity (as a percentage of sucrose equivalent content) and develop seedlings at different concentrations of PEG 6000, upper maternal lines.

Figures 5-6, only plant 1 from 7/3 combination registered a optical activity and seedlings survived at concentration of 20% PEG 6000 while maternal line (M7) showed optical activity only at 10% PEG 6000, although seedlings survived to 15% PEG 6000 concentration.

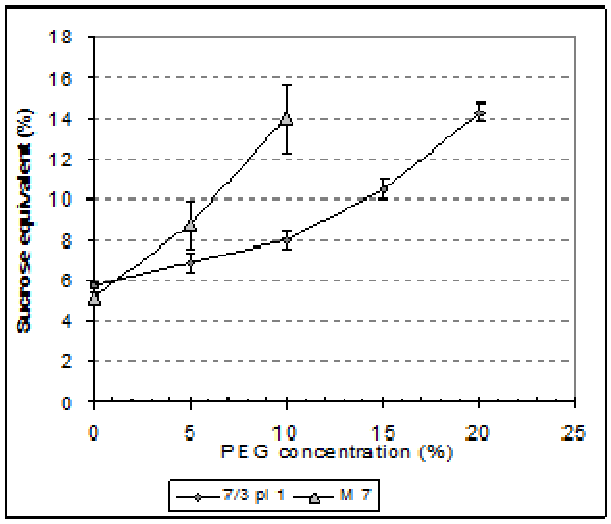

Figure 5. Optical activity for 7/3 plant 1 backcross 7 and maternal line M7.

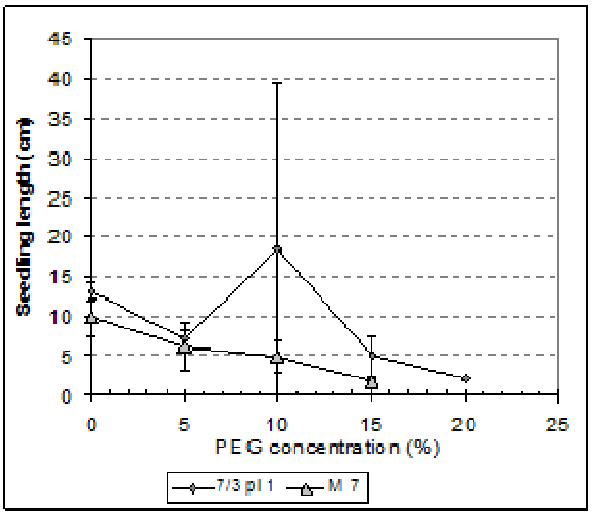

Figure 6. Seedling lenght for 7/3 plant 1 backcross 7 and maternal line M7.

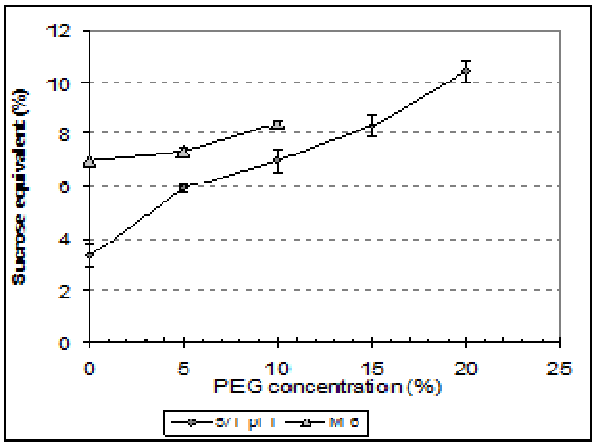

Figure 7. Optical activity for 5/1 plant 1 backcross 7 and maternal line M6.

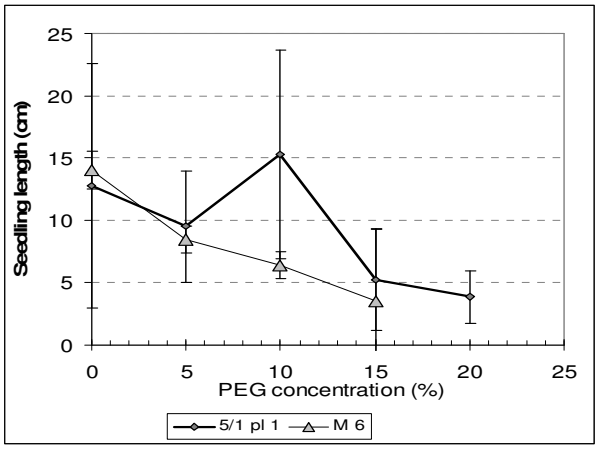

Figure 8. Seedling lenght for 5/3 plant 1 backcross 7 and maternal line M6.

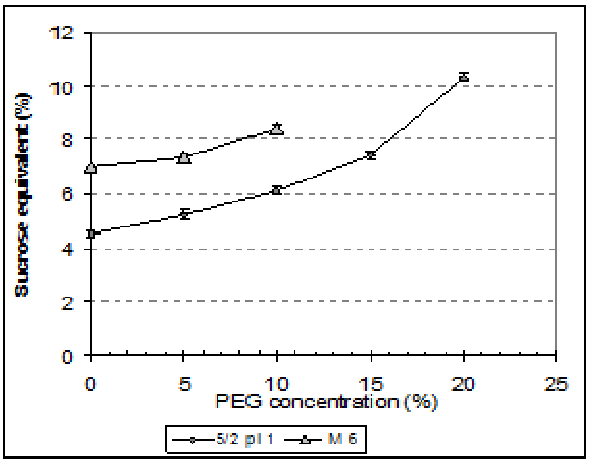

Figure 9. Optical activity for 5/2 plant 1 backcross 7 and maternal line M6.

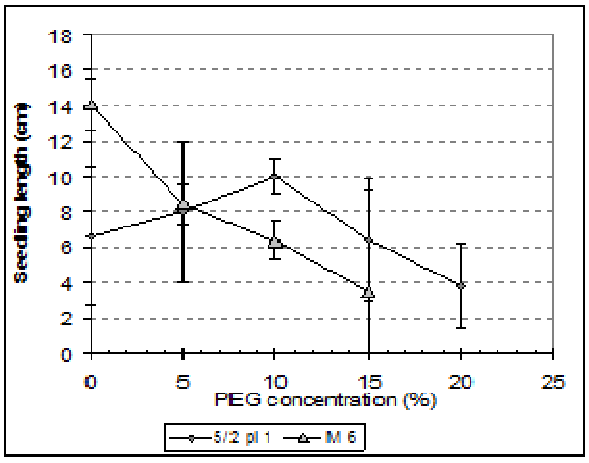

Figure 10. Seedling lenght for 5/2 plant 1 backcross 7 and maternal line M6

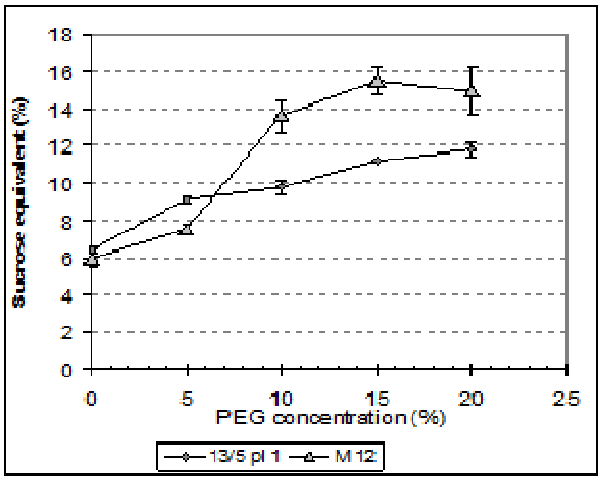

Figure 11. Optical activity for 13/5 plant 1 backcross 7 and maternal line M12.

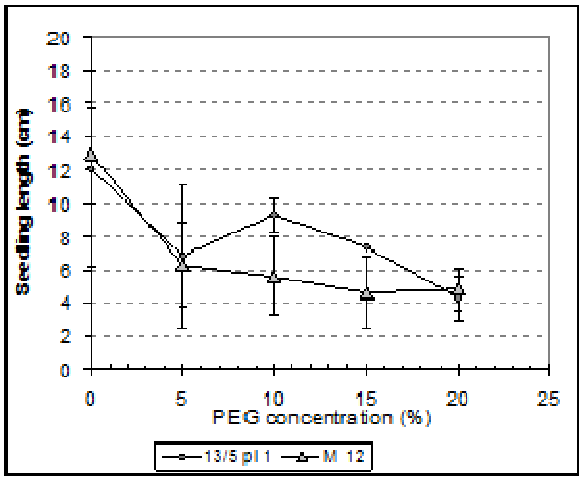

Figure 12. Seedling lenght for 13/5 plant 1 backcross 7 and maternal line M12

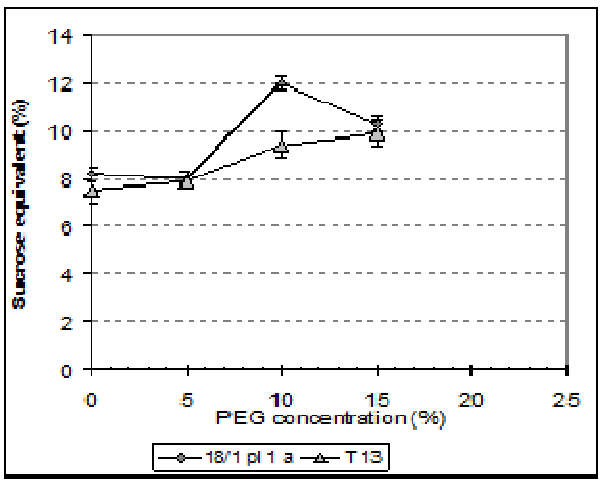

Figure 13. Optical activity for 18/1 plant 1a backcross 7 and paternal line T13

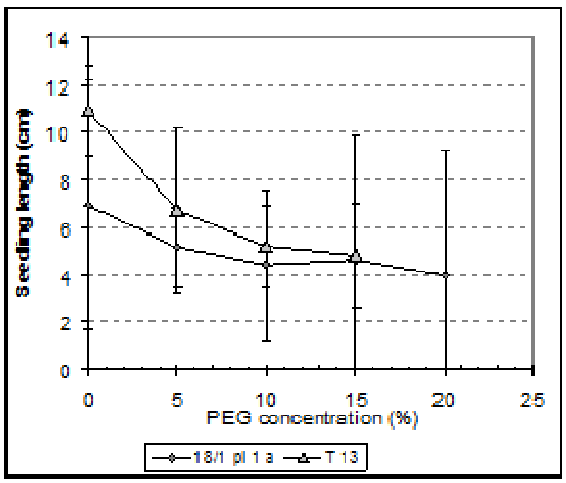

Figure 14. Seedling lenght for 18/1 plant 1a backcross 7 and paternal line T13

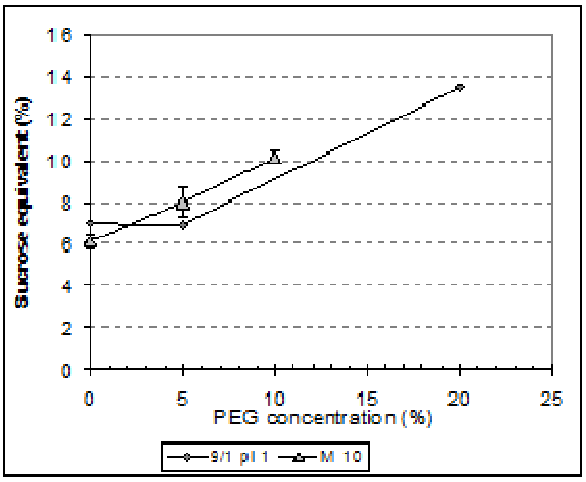

Figure 15. Optical activity for 9/1 plant 1 backcross 7 and maternal line M10

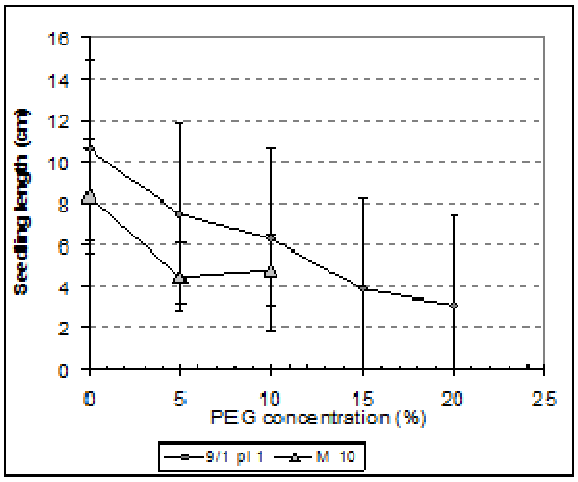

Figure 16. Seedling lenght for 19/1 plant 1 backcross 7 and maternal line M10

Figures 7-16 highlight the behaviour of individual plants belonging hybrid combinations showed high resistance to forms of maternal / paternal (T13), both in terms of optical activity of cellular juice but also in terms of length plantlets developed in conditions of water stress induced by PEG 6000. Thus genotypes: plant 1 from combination 5/1 and plant 2 from 5/2 (which have common maternal line M6) (Figure 7-8); plant 1 from 13/5 (with maternal line M12) (Figure 9-10); plant 1 from 18/1 (expressing anthocyanic and branched characters obtained from male line T13) (Figure 11-12); plant 1 from 9/1 (maternal line M10) (Figure 13-14). Certainly they will be used in the final stage of improving for drought resistance, in order to obtain new hybrids.

CONCLUSIONS

For 68% of studied lines were pointed out backcross descendents that exceed the optical activity of their maternal lines at least for one of the tested PEG 6000 concentrations. For the length of seedlings this percentage was 50%. The 10% PEG 6000 concentration permitted the best differentiated of the tested material and it will be maintained in our further studies. The contribution of the *Helianthus argophyllus* genotype permitted a better growth and survival of the seedlings from backcross 7 in the higher tested concentrations (15 and 20 % PEG). In many cases it was noticed that related genetic combinations had a similar response of juice concentration and growth to osmotic stress. Standard deviations for optical activity were relatively lower than those for seedling length. This preliminary study will be continued with the analysis of the changes of O.A. in time and correlation with the yield data from field trials.

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