SCREENING OF SOME SUNFLOWER HYBRIDS FOR DROUGHT TOLERANCE UNDER LABORATORY CONDITIONS

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Abstract

Helianthus annuus L. is one of the most important crops in the world, widely cultivated for its edible seeds and high-quality oil with a wide range of application in human consumption, oleochemical, pharmaceutical and cosmetics industries and also for biodiesel production. In the environment with changing climatic conditions the global agricultural production (including sunflower seeds) suffers considerable decrease due to the negative impact of abiotic factors such as heat, drought, salinity etc. Therefore, one of the main objectives of the breeders is to obtain drought tolerant varieties, which can express their full yield potential inclusively under drastic water deficiency. Though sunflower is considered drought-tolerant crop, it is very sensitive to water deficit at the stage of germination, seedling and flowering. In this context, the aim of this study was to evaluate drought tolerance potential in some new sunflower experimental hybrids at germination and early seedling growth stages under laboratory conditions using PEG-6000. Two levels of osmotic stress (induced by PEG concentrations 10 and 20%) were created and the reaction of genotypes was evaluated against a control. Germination rate (GR), germination stress tolerance index (GSI), plant height stress index (PHSI), root length stress index (RLSI) and dry matter stress index (DMSI) were used to determine the genotypic response to water stress. Drought stress affected the germination rate and seedling traits in all samples, indicating significant differences among genotypes and PEG concentrations. Plant height and seedling fresh weight are the most affected, such as a significant reduction of these parameters was observed in all sunflower genotypes. In contrast, an increase in RLSI was observed in the majority of tested sunflower hybrids. The hybrids HM1, HM11, HM14, HM17 and HM20 created by Moldavian Company AMG-Agroselect Comert and H5, H9 and H17 belonged from NARDI Fundulea, Romania performed well among evaluated sunflower genotypes, indicating a greater tolerance in terms of germination rate, shoot and root development, as well as dry plants weight at both levels of water stress. The genotypes with best performance could be recommended for breeding program and cultivation in areas with water deficiency during the germination and early seedling growth stages.

Key words: sunflower, drought stress, PEG, germination, seedling

Sunflower (*Helianthus annuus* L.) is one of major crops of global importance. Grown on about 23 million hectares, with annual production of 40 million metric tons it is the fifth largest oilseed crop in the world (www.fas.usda.gov). Traditionally, sunflower seeds have been grown especially for human consumption (oil/meal or dried product in bakery, as a snack), but in the context of the recent developments in EU and US biofuel policies, sunflower became popular inclusively as a significant biofuel feedstock (http://agritrade.cta.int).

In the environment with changing climatic conditions the global agricultural production (including sunflower seeds) suffers considerable decrease due to the negative impact of a series of abiotic and biotic factors. The abiotic stress such as heat, drought, salinity have could reduce average yields for most major crop plants by >50% (Wheaton E. *et al*, 2008).

Based on climatic trends observed in recent years, characterized by an increase in temperature and decrease of relative humidity, as well as future prediction, a potential decrease in sunflower production of around 10-30% is expected for European countries (Debaeke Ph. *et al*, 2017). Climate changes affect especially developing and transition countries. Thus, according to World Bank report on rural productivity in the Republic of Moldova, the annual losses from drought were estimated at US $20 million per/year. Losses caused by other

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events like severe weather and floods were estimated to approximately US $7.5 million and US $5 million. The severe drought of 2007 caused significant losses for the agricultural sector estimated at over EUR 1 billion (http://siteresources.worldbank.org). Therefore, one of the most important aims of the breeders is to obtain drought tolerant varieties/hybrids, which can express their full yield potential inclusively under drastic water deficiency.

Although sunflower is considered moderately drought tolerant crop due to its long and deep root system (Hussain S.S. *et al*, 2016), it is very sensitive to water deficit at some developmental stages. Thus, plants are significantly affected by the drought at the stage of germination, seedling and flowering (Ahmad S. *et al*, 2009). Seed germination is one of the most critical and sensitive to the changes of environmental conditions phases of development. Drought stress may reduce or delay seed germination and compromise the seedlings establishment (Albuquerque F.M.C. and de Carvalho N.M., 2003; Li H. *et al*, 2013). Also, under stress conditions different seedling particularities (volume and lengths of root, lengths of shoot and coleoptiles, dry and fresh weight) are significant affected (Ahmad S. *et al*, 2009; Saensee K. *et al*, 2012; Chachar Z. *et al*, 2016).

An efficient and rapid way for the primary screening to drought tolerance under laboratory is the application of polyethylene glycol (PEG 6000) in order to create osmotic stress consequences of drought stress condition. Many studies on plant responses to PEG induced drought stress with regard to seed germination and seedling growth have been reported in different crops, including sunflower (Geetha A. *et al*, 2012; Ghebremariam K.M. *et al*, 2013; Kaya M.D. *et al*, 2006; Sauca F. *et al*, 2017; Toscano S. *et al*, 2017).

The main objective of this study was to evaluate drought tolerance potential in some new sunflower experimental hybrids created in Romania and Republic of Moldova at germination and seedling stages under laboratory conditions, using PEG-6000.

MATERIAL AND METHODS

For the study were used 40 experimental sunflower hybrids, including 20 hybrids (noted conventionally HM1-HM20) created by the Company AMG-Agroselect Comert, Republic of Moldova and 20 hybrids (noted as H1-H20) created by the National Agricultural Research and Development Institute – Fundulea, Romania. The plants were tested against drought stress at germination and seedling stages under laboratory conditions (25±3°C) in Petri dishes for 10 days.

Polyethylene glycol with a molecular weight of 6000 (PEG-6000) in two concentrations (10% and 20%) was used to induce drought stress. The seeds of each sunflower genotype were soaked twice with 70% ethanol, surface sterilized with 10% sodium hypochlorite solution for 10 min and washed with distilled water several times. Seeds of each sunflower genotype (twenty for each replication) were placed in Petri plate containing 2-layers of Whatman filter papers. Three replications of seeds were treated with 10,0% solution of PEG 6000 in distilled water, other 3 replications by 20,0% solution of PEG and in the last group of samples (control) distilled water was used. Five mL of PEG solution or distilled water were added in each Petri plate when required.

Number of seeds germinated was counted daily and data were recorded for 10 days. After 10 days, the numbers of germinated seeds were recorded and the germination rate (GR), promptness index (PI) and germination stress index (GSTI) was calculated according to George (1967), using following formulae:

GR = Germinated seeds / Total Seeds x 100;

PI = nd2(1.0)+nd4 (0.75)+nd6 (0.50)+nd8 (0.25), where: nd2, nd4, nd6, nd8, represent the number of germinated seeds at 2nd, 4th, 6th, and 8th day, respectively.

GSTI = (PI of stressed seeds /PI of control seeds) x 100.

The shoot and root length was measured in centimeter with ruler after 10 days of the start of the experiment. Shoots and roots were separated and weighed in grams (g). Plant dry weights were recorded after drying at 70°C to a constant weight. From these measurements the plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI) and dry matter stress tolerance index (DMSI) were calculated according to Ashraf M.Y. *et al,* (2006), as following:

PHSI = (Plant height of stressed plant / Plant height of control plants) x 100

RLSI = (Root length stressed plant / Root length of control plants) x 100

DMSI = (Dry matter of stressed plant / Dry matter of control plants) x 100.

The experimental data were statistically analysed according to Dospekhov B. (1985).

RESULTS AND DISCUTION

Analysis of the effect of PEG induced drought stress on the germination and seedling growth showed a significant reduction in germination percentage and plant growth parameters in all tested sunflower genotypes.

The highest germination rate (GR) was observed in absence of drought stress (control), especially in the group of sunflower hybrids belonged from the Republic of Moldova (*figure*

1). The GR ranged between 75% and 100%, comparative to those shown by hybrids created in Romania – 16.7-76.7%. The highest value (more than 95%) of GR in the first group was observed in HM1, HM3, HM8-HM11, HM13-HM17, HM19 and the lowest values were revealed in genotypes HM12 (75%) and HM18 (80%).

In the hybrids belonged from Romania the maximal values of germination rate (65.0-76.7%) were established in hybrids H6, H10, H14 and H17, with maximum in H14, while the minimal values (less than 30%) were established in H11, H15, H16 and H20. Sunflower hybrids H7, H8, H12 and H13 didn't germinate in the conditions of experiments.

Polyethylene glycol reduced significantly the seed germination and GR decreased as PEG concentration increased (*figure 1*). The minimum GR values were recorded at the highest 20% PEG concentration both in Romanian and Moldavian sunflower hybrids, with lowest value in Romanian hybrids. Thus, in H1, H3, H11, H15, H16, H18 and H20 GR ranged between 1.67-16.67%, comparative with the control values 16.7-51.5% in the same genotypes (data does not show).

In the hybrids created by AMG-Agroselect Comert the GR varied between 71.67-98.3 under the samples stressed by 10% of PEG and 36.67-86.67% in those treated with 20% of PEG, with lowest values in HM2 (43.3%), HM3(50.0%), HM5 (36.67%), HM12 (48.3%) and HM18 (42.5%). A similar trend in both experimental groups was observed inclusively in the case of 10% PEG induced water stress. Reduced seed germination rate is in agreement with the results reported by Kaya M.D. *et al* (2006) in sunflower genotypes.

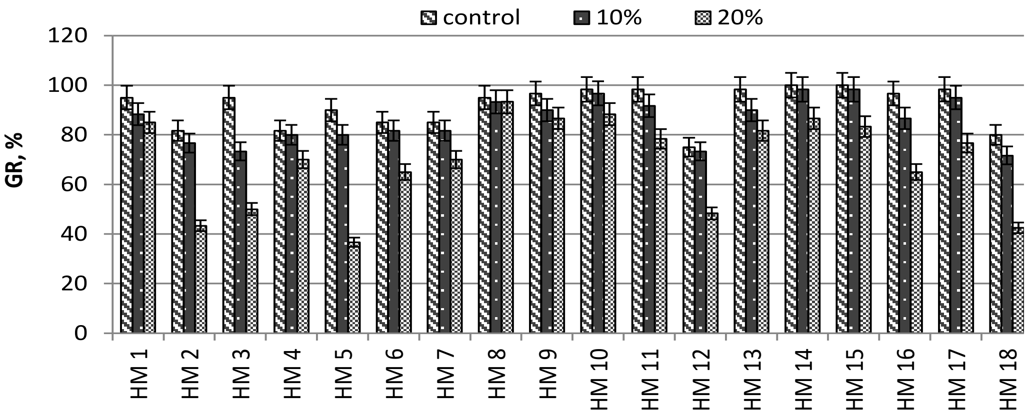

Figure 1 **Effect of different PEG concentrations on final germination rate (GR) of some sunflower hybrids.**

The germination stress tolerance indices (GSI) values ranged from 57.39% (HM3) to 99.76% (HM8) under the lowest osmotic potential (10% PEG) compared to 32,00% (HM18) – 93.84% (HM8) at higher osmotic potential (20% PEG). Around 70% of Moldavian sunflower hybrids shown high values of GSI (more than 90%) at 10% PEG and around 30% of analysed samples presented values of GSI higher than 80% at the greatest concentration of PEG (*figure 2*). The hybrids HM1, HM8, HM10, HM11, HM 13-HM15, HM17 and HM20 showed high GSI value under both tested PEG concentrations, while sunflower hybrids HM2, HM3 and HM19 showed minimum germination stress tolerance indices.

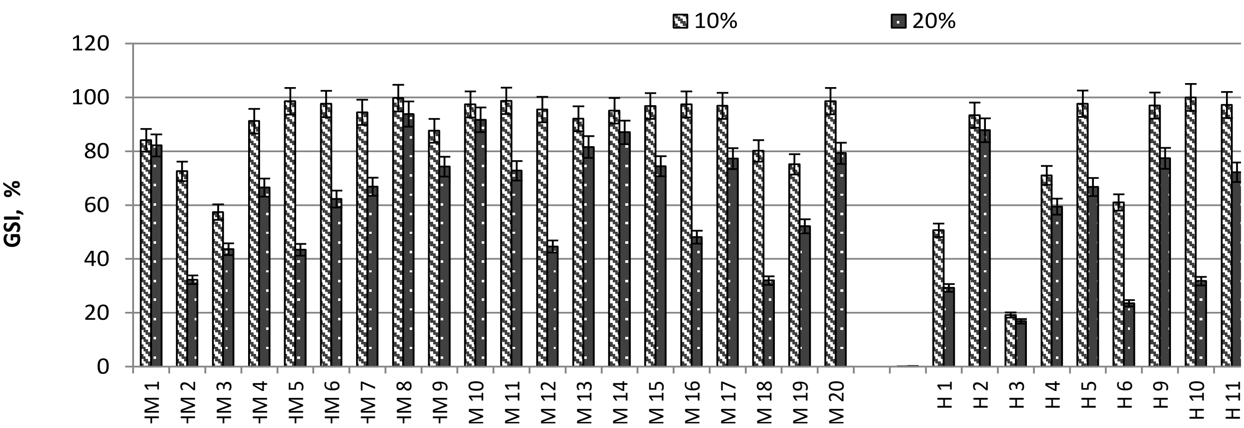

Figure 2 **Effect of different PEG concentrations on germination stress tolerance index (GSI) of some sunflower hybrids belonged from the Republic of Moldova (HM) and Romania (H).**

The GSI values in hybrids H1-H20 ranged between 19.13-100.0% under 10% of PEG and 16.86-87.82% under 20% of PEG (*figure 2*). In the majority (62,5%) of sunflower hybrids belonged from this group the GSI value exceed 90.0% at lowest PEG concentration. The highest GSI value was 100% and 99.06% for hybrids H10 and, respectively, H19 and the lowest was 19.13% in sample H3. At 20% PEG, the highest value 93.4% was revealed in H2 and the lowest 16.86% in H3. The maximal GSI values in both PEG concentrations were recorded in H2, H5, H9, H11 and H19 and the minimal – in the samples H1, H3 and H18.

Drought stress affected the germination rate in all tested sunflower genotypes indicating significant differences among genotypes and PEG concentrations. The results were in agreement with those obtained by Ahmad S. *et al* (2009) and Saensee K. *et al* (2012) in some sunflower hybrids/synthetic varieties under PEG induced water stress, as well as with the data reported by Ghebremariam K.M. *et al* (2013) in tomato and by Chachar Z. *et al* (2016) in wheat. Ayaz F.A. *et al* (2000) concluded that decrease of seed germination and seedling growth under osmotic stress is due by the disturbance of metabolic process leading to increase in phenolic compounds. Water deficiency affects cell division and plant growth metabolism causing the delay in seedling emergence and yield decrease.

The physiological indices such as height stress index (PHSI), root length stress index (RLSI) and dry matter stress index (DMSI), also were used to evaluate the response of sunflower hybrids to PEG-induced water stress. A significant reduction in shoot length was observed in all sunflower genotypes. Thus, the plant height stress tolerance index (PHSI) decreased in both tested PEG concentrations (*figure 3*).

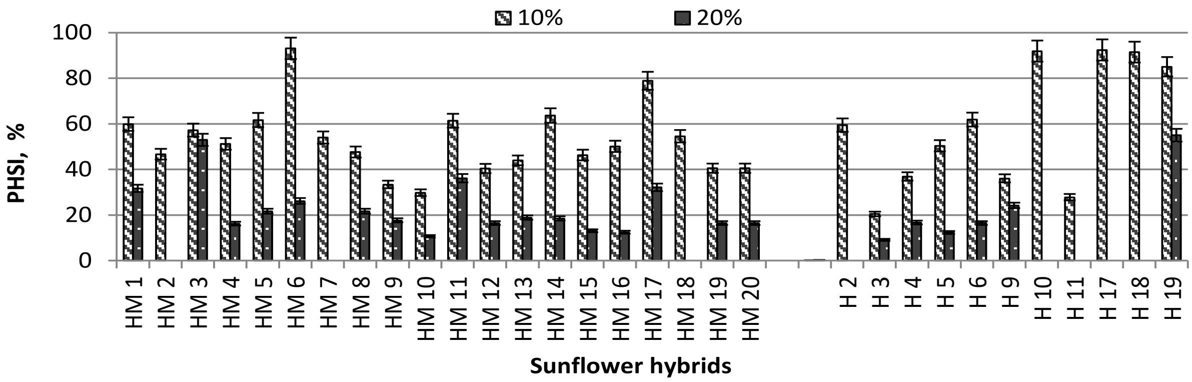

Figure 3 **Effect of different PEG concentrations on plant height stress tolerance index (PHSI) of some sunflower hybrids belonged from the Republic of Moldova (HM) and Romania (H).**

The highest PHSI values at 10% PEG was 93.1% for sunflower hybrid HM6, followed by the genotypes HM17 (78.89%), HM14 (63.64%) and HM11 (61.37%). In the samples treated with 20% of PEG solution the maximal values of PHSI was 52.99% in HM3, 36.14% in HM11 and 32.16% in HM17. The lowest value under both stress levels was revealed by HM10 (29.77% and, respectively, 10,70%).

In the case of sunflower hybrids belonged from Romania characterized by lower germination rate these parameters could be ranked only for nine genotypes (H3-H11, H17 and H18). The greatest PHSI value (92.35%) was observed in H17 followed by H10 (91.88%), while minimum value (20.45%) was recorded in H3 (*figure 3*).

At 20% PEG concentration PHSI values were significantly lower than in first experimental group, ranging between 9.09-24.16%, with minimum in H3 and maximum in H9. Generally, it has been established that shoot length decreased with drought levels increased. The results are in agreement with findings of previous studies in sunflower (Ahmad S. *et al*, 2009; Moghanibashi M. *et al*, 2012; Saensee K. *et al*, 2012; Toscano S. *et al*, 2017).

Water deficiency also significantly affected the root length (*figure 4*). Results showed that in the conditions of moderate drought stress root growth was faster than in control, but the radicel were very thin and delicate. Thus, at 10% PEG treatment in Moldavian hybrids HM4, HM5, HM11, HM14, HM16 and HM17 the RLSI values varied between 126.85-265.85%, with maximum in HM16. Sunflower hybrids HM11 and HM17 maintained the highest value of RLSI (113.76 and 120.20%) under 20% PEG concentration. In the second experimental group the RLSI exceed 100% only in hybrid H5.

Root elongation declined by increasing PEG concentration. The lowest RLSI values were revealed in HM3 (48.11% and 26.07% under 10% and, respectively, 20% PEG concentration) and H3 (46.43% and 42.86%, respectively).

According to Sharp R.E. and Davies W.J. (1989) the development of the roots under water stress are usually less inhibited than growth of shoots and may be even stimulated. This trait is under genetic control and constitutes a benefit to maintain an adequate water supply in plant (Sponchiado B.N. *et al*, 1989). Thus, higher root growth is linked with better drought tolerance.

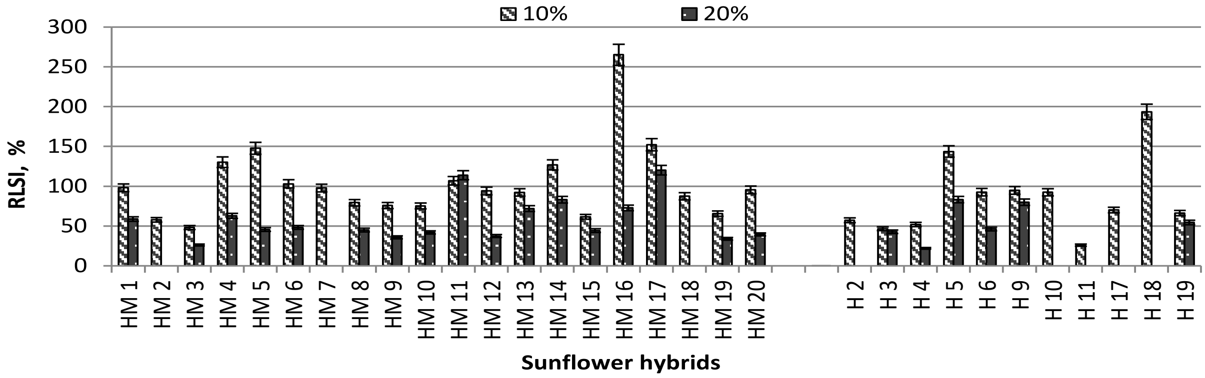

Figure 4 **Effect of different PEG concentrations on root length stress tolerance index (RLSI) of some sunflower hybrids belonged from the Republic of Moldova (HM) and Romania (H).**

It was not observed any significantly differences between control and experimental samples in HM1, HM7, HM12, HM13, HM 18, HM20, H6, H9 and H10, when the RLSI varying between 92.09-97.96%.

Dry matter stress tolerance index (DMSI) decreased significantly with the increase in PEG concentration (*figure 5*). The lowest DMSI values were revealed among sunflower hybrids belonged from Romania. The minimum values were recorded in H3 (10.31%) and H11 (19.26%) at 20% PEG concentration. In the case of samples treated with 10% PEG solution, the lowest values (28.89-54%) were recorded in H5, H6, H9, H11. In this group, maximum DMSI values (90%) was established under 10% PEG in H4, followed by H17 (68%).

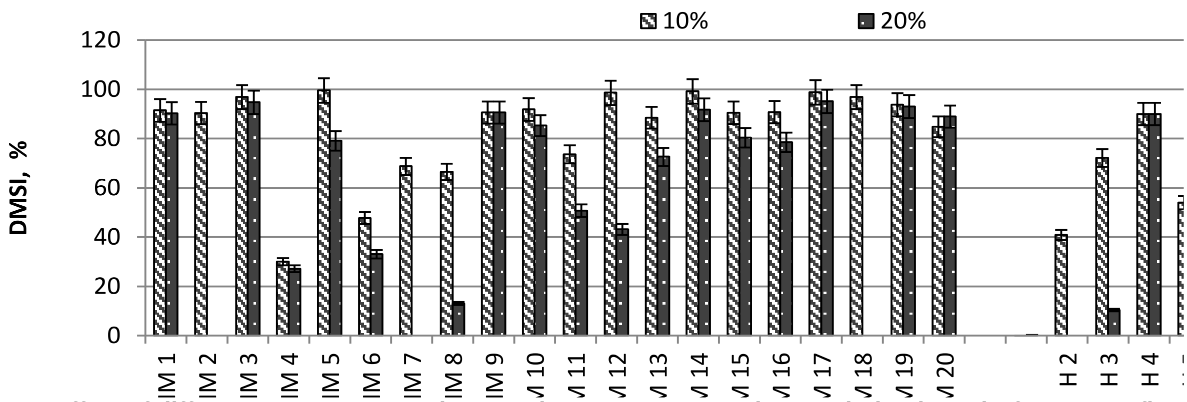

Figure 5 **Effect of different PEG concentrations on dry matter stress tolerance index (DMSI) of some sunflower hybrids belonged from the Republic of Moldova (HM) and Romania (H).**

Moldavian hybrids were less affected (*figure 5*). Thus, in majority of analysed genotypes (HM1, HM3, HM5, HM9, HM10, HM14-HM17, HM19, HM20) DMSI ranged between 78.47-99.56% in both PEG concentrations. The maximum values for DMSI were observed under 10% PEG application. Hybrids HM1, HM3, HM9, HM14, HM17 and HM19 maintained the highest dry matter stress tolerance index (more than 90%) even under 20% PEG treatment. Notable that since seedling dry weight in these genotypes did not change significantly, the fresh weight decreased with the increasing of PEG concentration, ranging between 21.73-72.79% to the control. Obtained data suggest that water stress induces only a decreasing of water content in seedling and doesn't influence the seedling mass accumulation.

The lowest values for DMSI was observed in genotypes HM4 (29.99% and 27.14% under 10% and, respectively, 20% PEG concentration) and HM6 (47.71% and 33.01%, respectively). The results are similar with those reported by Ahmad S. *et al* (2009), Geetha A. *et al* (2012) and Saensee K. *et al* (2012).

CONCLUSIONS

The present study showed that the germination and seedling traits of sunflower genotypes are significant affected by water stress. The exposure of plants to two levels of water deficit induced by different concentrations (10% and 20%) of PEG-6000 solution, led to differential GR, GSI, PHSI, RLSI and DMSI response in analyzed genotypes and it can be concluded that this method is reliable, cheap, rapid and could be used for sunflower screening. Drought stress affected especially plant seedling growth, such as a significant reduction in shoot length was observed in all sunflower genotypes. Changes in the plant height could be considered as a sensitive and useful indicator of drought stress.

The hybrids HM1, HM11, HM14, HM17 and HM20 created by Moldavian Company AMG-Agroselect Comert and H5, H9 and H17 belonged from NARDI Fundulea, Romania performed well among evaluated sunflower genotypes, indicating a greater tolerance in terms of germination rate, shoot and root development, as well as dry plants weight at both levels of water stress. Also, these genotypes showed high productivity indices in field experiments (Tabara O. *et al*, 2018).

The hybrids with best performance could be recommended for breeding program and cultivation in areas with water deficiency during the germination and early seedling growth stages.

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REFERENCES

Ahmad S., Ahmad R., Ashraf M.Y., Ashraf M., Waraich E. A., 2009 – *Sunflower (Helianthus annuus l.) Response to drought stress at germination and seedling growth stages*. Pak. J. Bot., 41(2):647-654.

Albuquerque, F.M.C., de Carvalho, N.M., 2003 – *Effect of type of environmental stress on the emergence of sunflower (Helianthus annuus L.), soybean (Glycine max (L.) Merril) and maize (Zea mays L.) seeds with different levels of vigo*r. Seed Sci. Technol. 31:465-467

Ashraf M.Y., Akhter K., Hussain F., Iqbal **J.**, **2006** – *Screening of different accessions of three potential grass species from Cholistan desert for salt tolerance*. Pak. J. Bot., 38(5): 1589-1597.

Ayaz F.A., Kadioglu A., Urgut R.T., 2000 – *Water stress effects on the content of low molecular weight carbohydrates and phenolic acids in Cienanthe setosa.* Canadian J. Plant Sci., 80:373-378

Chachar Z., Chachar N. A., Chachar Q.I., Mujtaba S.M., Chachar G.A., Chachar S., 2016 – *Identification of drought tolerant wheat genotypes under water deficit conditions*. International Journal of Research, 4(2):206-214.

Debaeke Ph., Casadebaig P., Flenet F., Langlade N., 2017 – *Sunflower crop and climate change: vulnerability, adaptation, and mitigation potential from case-studies in Europe.* OCL, 24(1) D102.

Dospekhov B., 1985 – Metodika polevogo opyta (s osnovami statisticheskoy obrabotki rezultatov issledovaniy) [The method of field experience (the basics of statistical processing of research results)]. Moscow: Agropromizdat Publ, 5th ed. 352 p.

Geetha A., Sivasankar A., Prayaga L., Suresh J., Saidaiah P., **2012** – *Screening of sunflower genotypes for drought tolerance under laboratory conditions using PEG*. Journal of Breeding and Genetics 44(1):28-41.

George. D. W. 1967. *High temperature seed dormancy in wheat (Triticum aestivum L*). Crop Sci., 7:249-253.

Ghebremariam K. M., Liang Y., Li C., Li Y., Qin L., 2013 – *Screening of tomato inbred-lines for drought tolerance at germination and seedling stage*. Journal of Agricultural Science, 5(11):93-101.

Hussain S.S., Ahsan M.A., Sornaraj P., Ali M., Shi B.J., 2016 – *Toward integration of a systems-based approach for understanding drought stress in plants*. In: Parvaiz A, ed. Water stress and crop plants: a sustainable approach. John Wiley & Sons, 227–247.

Kaya M.D., Okcu G., Atak M., Cikili Y., Kolsarici O., 2006 – *Seed treatments to overcome salt and drought stress during germination in sunflower (Helianthus annuus L.).* Eur. J. Agron., 24:291–295.

Li H., Li X., Zhang D., Liu H., Guan K., 2013 – *Effects of drought stress on the seed germination and early seedling growth of the endemic desert plant Eremosparton songoricum (Fabaceae*). EXCLI J.,12:89–101.

Moghanibashi M., Karimmojeni H., Nikneshan P., Behrozi D., 2012 – *Effect of hydropriming on seed germination indices of sunflower (Helianthus annuus L.) under salt and drought conditions*. Plant Knowledge Journal, 10-15.

Saensee K., Machikowa T., Muangsan N., 2012 – *Comparative performance of sunflower synthetic varieties under drought stress.* Int. J. Agric. Biol., 14(6):929-934.

Saucă F., Anton F.G., 2017 – *Screening of some sunflower genotypes (Helianthus annuus) for drought stress using PEG 6000*. Merit Research Journal of Agricultural Science and Soil Sciences, 5(3):054-059.

Sharp R.E., Davies W.J., 1989 – *Regulation of growth and development of plants growing with a restricted supply of water*. In: Jones HG, Flowers TL, Jones MB, eds. Plants under stress. Cambridge: Cambridge University Press, 71–93.

Sponchiado B.N., White J.W., Castillo J.A., Jones P.G., 1989 – *Root growth of four common bean cultivars in relation to drought tolerance in environments with contrasting soil types.* Experimental Agriculture, 25:249–257.

Tabără O., Rîșnoveanu L., Gîscă I., Clapco S., Joiţa-Păcureanu M., Duca M., 2018 – *Evaluarea unor hibrizi de floarea-soarelui privind rezistența la secetă în Republica Moldova și România.* Revista Știința Agricolă, 2:3-15.

Toscano S., Romano D., Tribulato A., Patanè C., 2017 – Effects of drought stress on seed germination of ornamental sunflowers. Acta Physiologiae Plantarum, 39(8), 1-12

Wheaton E., Kulshreshtha S., Wittrock V., Koshida G., 2008 – *Dry times: hard lessons from the Canadian drought of 2001 and 2002*. Can. Geograph., 52:241–262.