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RESEARCH ARTICLE

DEVELOPMENT OF BEST SCREENING METHOD AT SEEDLING STAGE UNDER DROUGHT STRESS FOR *BRASSICA NAPUS* L.

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ABSTRACT

Drought stress is the most crucial factor among different abiotic stresses that limits crop production and its growth worldwide than any other stress. Screening of available germplasm is the basic tool for plant breeding. But unfortunately, there is no well-developed screening method of brassica. For this purpose, ten accessions of *Brassica napus* were grown in hydroponics and *in vitro* culture. For hydroponics, seeds of each accession were sown in polythene bags Fifteen days after sowing, seedlings were transplanted to the hydroponics at three drought stress levels T_0= 0 (control), T_1= 9% and T_2= 18% of polyethylene glycol. Data were recorded on various seedling parameters after 15 days of transplantation. For *in vitro* culture seeds were grown in MS zero media and explants were used for callus culture. Three drought stress treatments of polyethylene glycol were applied which were used in previous experiment. Various drought tolerant related characters were measured after 21 days of callus formation. Recorded data of both experiments were subjected to analysis of variance and multiple comparison test. From this study it was concluded that most of the traits showed highly significant differences among the accessions in hydroponic than in *in vitro* culture. So, this study is very helpful in the next breeding program for the screening of brassica lines under drought stress.

KEYWORDS

Water scarcity, Seedling parameters, Hydroponic culture, *In vitro* culture and polyethylene glycol-6000.

1. INTRODUCTION

Any factor that causes reduction in crop yield either in deficient or excess form is called stress. Drought stress is the most crucial factor among different abiotic stresses that limits crop production and its growth worldwide than any other stress [1-4]. Drought effects on plants adversely. It reduces uptake of nutrients by roots, photosynthetic activity, canopy absorption of photosynthetic radiations, radiation use efficiency and harvest index. Plants manage drought stress through various approaches of stress avoidance and tolerance. Unfortunately, water scarcity has also bad effect on the *Brassica napus* particularly at seedling stage [5,6]. Its physiological and metabolic functions significantly reduce under water scarcity. Drought is also responsible for late seedling establishment, delayed or inhibition of seed germination. When water potential between seed and external environment is decreased, emergence of radicle is also inhibited [7]. Seeds sown having unfavorable soil moisture at the time of sowing results poor emergence of seedlings. In limited moisture supply, final crop yield decreases [8,9].

In any breeding program screening from available germplasm is a basic step. Genetic differences and similarities that exist in the genotypes are efficiently utilized as genetic resource. Various methods have been used by plant breeders to screen the drought tolerant accessions. But soil heterogeneity, labour, huge plant material, time and uncontrolled conditions make experiments of screening more difficult. Controlled conditions can be maintained in glass house, green house, hydroponics, *in vitro* and laboratory.

Hydroponic technique is also called soil-less culture. The “hydroponic” word is derived from the Greek words “hydro” means water and “ponos” means labor. It is a soil-less culture and plant roots are immersed in nutrients solution [10]. In hydroponics plant growth rate is 30-50% faster than plants grown in soil [10]. Plant yield is also greater in hydroponic and extra oxygen helps to stimulate the growth of roots. Artificial application of nutrients and supply of oxygen provide uniform conditions. It is also being used for screening of accessions under stress conditions.

Aseptic culture of tissues, organs, cells or plantlets under defined physical and chemical conditions is called as *in vitro*. *In vitro* culture is used to screen stress tolerant plants [11]. For development of stress tolerant cultivars this technique has been effectively used. *In vitro* selection depends on development of reliable and efficient callus induction and systems for plant regeneration for tolerance to abiotic stress. *In vitro* culture plays important role in understanding genetic processes of plants in short period of time and efficient way under controlled environment. It has attracted interest over last few years by providing different means to study physiological aspects and genetic processes. It also offers the potential for improved cultivars breeding by using genetic variability [12]. Various drought simulators are used to create the drought condition in lab experiments e.g. polyethylene glycol, mannitol, sorbitol etc. Polyethylene Glycol (PEG-6000) is the most appropriate drought simulator due to the higher molecular weight and non-penetrable. It is also best to create the dryness because seed germination percentage is approximately equal in soil and PEG-6000 mediated drought stress with the same potential of water [13].

Considering these points in view, present study was planned to find out the best screening method and also drought indicators in *Brassica napus* L. Genetic differences for drought related parameters among various *Brassica napus* accessions were also determined.

2. MATERIAL AND METHODS

Ten accessions of *Brassica napus* (B-56, B-18, ZMR-4, ZM-21, KM-256, ZMR-10, Punjab Sarsoon, Cyclon, Rainbow and UAF-11) were collected from Oilseed Research Group of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad and evaluated for drought stress at seedling stage in hydroponics and *in vitro* conditions. Hydroponic culture was conducted in wire house in the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Seven seeds of each accession per replication per treatment was grown in polythene bags with triplicate Completely Randomized Design (CRD) using factorial structured treatments. After 15 days of sowing, randomly selected five seedlings of each accession per replication per treatment were shifted to hydroponic solution developed by Hoagland and Snyder in 1933 called Hoagland solution at three drought stress levels T_0= 0 (control), T_1= 9% and T_2= 18% of polyethylene glycol (PEG-6000). Data were recorded on seedling height (cm), root and shoot length (cm), fresh and dry shoot weights (g) and fresh and dry root weights (g) after 15 days of stress application. Stress indices for drought stress treatments of each trait were calculated. Stress indices for each character also computed by using formula:

Stress index (SI) = (Stressed plant/ Control plant) × 100

In vitro culture was conducted in Centre of Agricultural Biochemistry and Biotechnology (CABB) University of Agriculture, Faisalabad. For this purpose, MS zero media was prepared containing MS salt with vitamins, sucrose and agar. Five sterilized seeds of each accession per replication were sown in prepared MS zero media. Explants from seedlings were cultured on full strength MS media supplemented with KNO_3 (Potassium nitrate), Myoinositol, Casein hydrolysate, BAP (Benzylaminopurine) and NAA (Nepthaline acetic acid). Polyethylene glycol was added in full strength MS media to create two drought stress levels i.e. T_1= 9% and T_2= 18% of PEG. Twenty petri plates per accession per replication per treatment were maintained. After callus formation, data on relative fresh weight of callus (g), electrolyte membrane leakage, proline content **(**µg**)**, glycine betaine**(**µg**)** and total soluble sugar contents**(**µg**)** were recorded. Recorded data of both experiments were subjected to analysis of variance and multiple comparison test.

Figure 1: Seedlings in hydroponic tubs after 15 days of stress application

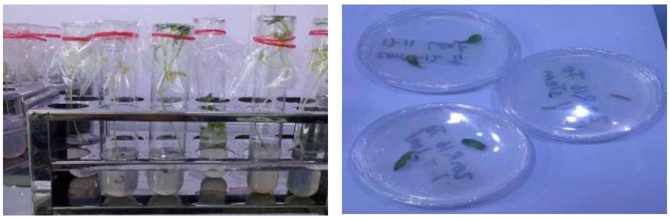

Figure 2: Callus culture of leaves of *Brassica napus* seedlings

3. RESULTS

Hydroponic Culture: The mean square values from analysis of variance of different drought stress indices are presented in Table 1. Treatments had significant differences for all stress indices except dry shoot weight and fresh root weight index.

Table 1: Mean square values of *Brassica napus* L. accessions for drought stress indices under drought stress treatments

SOV	DF	SHI	SLI	RLI	FSWI	DSWI	FRWI	DRWI
Treatments (T)	1	405.847*	2319.26*	8923726*	164237*	8407	196347	729301*
Accessions (A)	9	717.170*	2166.22*	131087	28164*	17861*	672859*	1026439*
T×A	9	232.211*	564.33*	132843*	18536	5151	191882*	395683*
Error	40	122.600	307.46	67735	11618	5984	88898	114286

*= significant at 0.05 probability level

SOV= Sources of variations, **DF**= Degrees of freedom, **SHI**= Seedling height index, **SLI**= Shoot length index, **RLI=** Root length index, **FSWI=** Fresh seedling weight index, **FRWI=** Fresh root weight index, **DSWI=** Dry shoot weight index and **DRWI=** Dry root weight index

Among accessions, all drought stress indices had significant differences except root length index while interaction of treatments and accessions had significant differences for all indices except fresh and dry shoot weight index (Table 1). A decreasing trend was observed for increasing the stress in *Brassica napus* accessions for seedling height index, root length index, fresh and dry shoot weight index and dry root weight index. Increasing trend was observed for shoot length index and fresh root weight index for increasing the stress. Index of T_1 had the maximum mean values for seedling height index (93.133), root length index (101.19), fresh shoot weight index (186.07) dry shoot weight index (91.588) and dry root weight index (377.48) while T_2 had maximum mean values for shoot length index (1036.5) and fresh root weight index (338.41) (Figure 3).

Results of mean comparison analysis showed that among accessions, seedling height index ranged from 76.55 to 110.24, root length index from 77.30 to 103.05, shoot length index from 313.91 to 719.24, fresh shoot weight index ranged 44.37 to 206.58, dry shoot weight index from 190.11 to 32.50, fresh root weight index from 107.6 to 1022 and dry root weight index from 66.3 to 1162.2 (Figure 4).

Accession ZMR-4 had maximum mean values for seedling height index (110.24), root length index (130.05) and fresh shoot weight index (206.58). Accession UAF-11 had maximum mean values for shoot length index (719.24), dry shoot weight index (190.11) and fresh root weight index (1022.0). Accession B-56 had maximum mean dry root weight index (66.3). Accession UAF-11 had minimum mean values for seedling height index (76.55) and root length index (77.30). Accession ZMR-10 had minimum dry shoot weight index (32.50) and fresh root weight index (107.6). Accession B-18, Cyclon and Rainbow had minimum mean shoot length index (313.91) fresh shoot weight index (44.37) and dry root weight index (66.3) respectively (Figure 4).

***In vitro* culture:** The mean square values from analysis of variance of different traits under normal and drought stress treatments are presented in Table 2. Treatments had significant differences for all the traits except total soluble sugar contents.

Table 2: Analysis of variance of *Brassica napus* L. accessions for different traits under control and drought stress treatments

SOV	DF	RFWC	EML	PC	GB	TSSC
Treatments (T)	2	0.14880*	0.66090*	0.10128*	0.00549*	0.04646
Accessions (A)	9	0.01221*	0.01744	0.04282	0.12551	0.04653
T×A	18	0.00242*	0.15204*	0.07490*	0.02610	0.07857*
Error	60	0.00078	0.02263	0.03467	0.03795	0.02874

*= significant at 0.05 probability level

SOV= Sources of variation, **DF=** Degrees of freedom, **RFWC**= Relative fresh weight of callus, **GB**= Glycine betaine, **PC=** Proline content, **TSSC=** Total soluble sugar contents and **EML=** Electrolyte membrane leakage

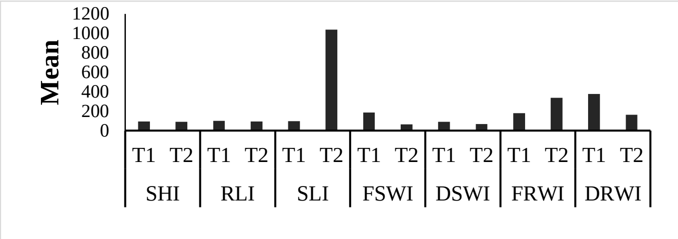

Figure 3: Mean comparison of drought stress indices of both trearments in *Brassica napus* L

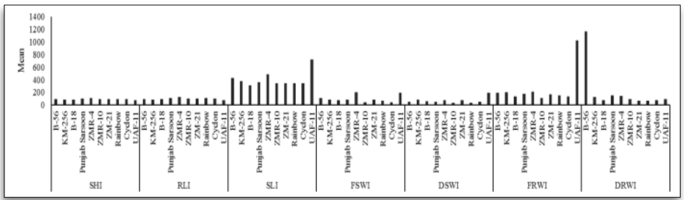

Figure 4: Mean comparison of *Brassica napus* L. accessions for different stress indices under control and drought stress treatments

SHI= Seedling height index, **SLI**= Shoot length index, **RLI=** Root length index, **FSWI=** Fresh seedling weight index, **FRWI=** Fresh root weight index, **DSWI=** Dry shoot weight index, **DRWI=** Dry root weight index, $\mathbf{T_1}$= 9% and $\mathbf{T_2}$= 18% of polyethylene glycol (PEG)

Accessions had significant differences for relative fresh weight of callus. All traits showed significant differences for interaction between treatments and accessions except glycine betaine (Table 2). A decreasing trend was observed for increasing the stress in *Brassica napus* accessions for relative fresh weight of callus. Glycine betaine and total soluble sugar contents showed random decreasing and increasing trend in accessions for increasing the stress. Increasing trend was observed for proline content and electrolyte membrane leakage for increasing the stress in *Brassica napus* accessions. T_0 had maximum mean values for relative fresh weight of callus (0.3270g), glycine betaine (0.3229µg) and total soluble sugar contents (0.5592µg) while T_2 had maximum mean values for proline content (0.3557µg) and electrolyte membrane leakage (0.5248) (Figure 5).

Results of mean comparison analysis showed that among accessions, relative fresh weight of callus ranged from 0.1946 to 0.3052, electrolyte membrane leakage from 0.2756% to 0.4046%, proline content from 0.1960µg to 0.3842µg, glycine betaine from 0.1878µg to 0.4222µg and total soluble sugar contents 0.4338µg to 0.6081µg (Fig. 6).

Accession Cyclon had maximum proline content (0.3842µg) and glycine betaine (0.4222µg). Accession ZMR-10, B-56 and B-18 had maximum relative fresh weight of callus (0.3052g), electrolyte membrane leakage (0.4046) and total soluble sugar contents (0.6081µg) respectively. Accession B-56 had minimum relative fresh weight of callus (0.1946g), proline content (0.1960µg) while Punjab sarsoon had minimum glycine betaine (0.1878µg) and total soluble sugar contents (0.4338µg). Accession Cyclon had minimum electrolyte membrane leakage (0.2756) (Figure 6).

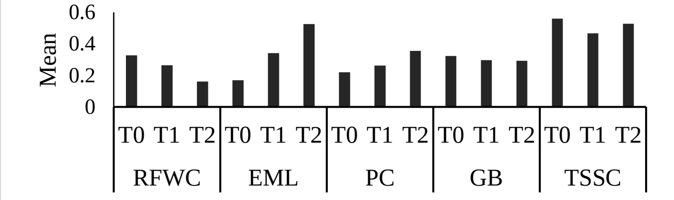

Figure 5: Mean comparison of control and drought stress treatments for different traits in *Brassica napus* L

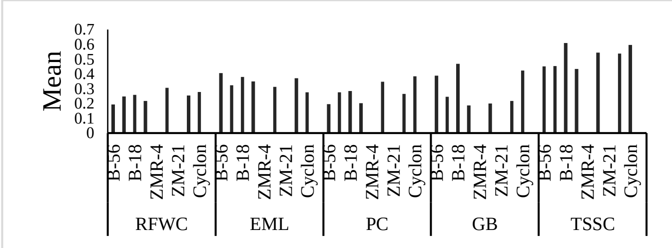

Figure 6: Mean comparison of *Brassica napus* L. accessions for different traits under control and drought stress treatments

RFWC= Relative fresh weight of callus, **GB**= Glycine betaine, **PC=** Proline content, **TSSC=** Total soluble sugar contents, **EML=** Electrolyte membrane leakage, $\mathbf{T_0}$= 0 (control), $\mathbf{T_1}$= 9% of PEG and $\mathbf{T_2}$= 18% of PEG

4. DISCUSSION

Results of hydroponic culture showed that seedling height index, root length index, dry root weight index and fresh shoot weight index decreased by increasing the drought stress while shoot length index, dry shoot weight index and fresh root weight index increased by increasing drought stress. Some group researchers reported gradual decreasing trend for drought stress indices [14,15]. This contradiction in results for shoot length index, dry shoot weight index and fresh root weight index may be due to different germplasm and environment. All the drought stress indices showed significant differences except dry shoot weight index and fresh root weight index among treatments, root length index among accessions and fresh shoot weight index and dry root weight index for interaction between treatments and accessions. Some other group researchers also reported significant differences for accessions, treatments and their interaction for different drought stress indices [5,16-19]. In *in vitro* culture, all the traits showed significant differences except total soluble sugar contents among treatments, and glycine betaine for interaction between treatments and accessions. In other study also reported significant differences among treatments and interaction between treatments and accessions [12,20,21]. Relative fresh weight of callus had decreased, and proline and glycine betaine had increased with increase in drought stress. Another group of researchers reported also decreasing trend in fresh weight of callus and increase in proline content under drought stress condition [22,23]. In other hand, a group researcher found increasing trend in glycine betaine in response to PEG [24,25].

In both hydroponic and *in vitro* culture polyethylene glycol (PEG) can be used as simulator of drought stress. Both are considered efficient techniques for screening. There is faster growth of plants in both than plants grown in soil. *In vitro* culture is a technique in which genetic processes of plants can be understand in short period of time. Fresh weights are greater in hydroponic because extra oxygen helps to stimulate the growth of roots. Hydroponic is a new phenotypic technique based on score of drought tolerance, fresh and dry weight of shoots and roots, lengths of shoots and roots and survivability of seedlings. In *in vitro* culture plants can be screened for drought tolerance on score of callus morphology, relative fresh weight of callus and measurement of biochemical parameters e.g. proline content, glycine betaine, electrolyte membrane leakage and total soluble sugar contents. Hydroponic culture is less expensive as compared to *in vitro* because the use of nutrients.

Results of both experiment showed that in hydroponics all the accessions showed growth under drought stress condition but in *in vitro* culture all the accessions did not show good germination. In hydroponic culture, accession ZMR-4 had maximum mean value for seedling height index, root length index and fresh shoot weight index. Accession UAF-11 had maximum mean value for shoot length index, dry shoot weight index and fresh root weight index. In *in vitro* culture, accession Cyclon showed maximum mean value for proline content and glycine betaine and minimum value for electrolyte membrane leakage. Accessions ZMR-10 and B-18 showed maximum mean value for relative fresh weight of callus and total soluble sugar contents respectively. Genetic variability showed that these accessions can be used as further breeding programs. Among treatments, T_2 (18% of PEG) showed maximum mean values for proline content and electrolyte membrane leakage under *in vitro* condition and T_1 (9% of PEG) showed maximum value for seedling height index, root length index, fresh shoot weight index, dry shoot weight index and dry root weight index.

5. CONCLUSION

In the present breeding material genetic variability may be exploited in the hybridization programs or for the development of stress tolerant types in future. Performance of accessions ZMR-4 and UAF-11 in hydroponic culture and ZMR-10 and Cyclon in *in vitro* culture for stress treatment trait under study suggests use for future programs of breeding and for the development of drought tolerant cultivars.

Hydroponics showed best results as it possesses genetic variability for most of the traits and high germination % among *Brassica napus* L. as compared to *in vitro* culture. It is also better for the study of roots and its extensions under drought stress conditions. Moreover, chances of contamination were less in hydroponics. It is also cheaper and less expertise are required to conduct the experiment. Field screening is relatively difficult due to soil heterogeneity so the screening of brassica lines in hydroponic conditions is very effective. So it may be used in the screening of various crops.

REFERENCES

[1] Zheng, J., Fu, J., Gou, M., Huai, J., Liu, Y., Jian, M., Huang, Q., Guo, X., Dong, Z., Wang, H., Wang, G. 2010. Genome-wide transcriptome analysis of two maize inbred lines under drought stress. Plant Molecular Biology, 72, 407-423.

[2] Almeselmani, M., Abdullah, F., Hareri, F., Naaesan, M., Ammar, M.A., Kanbar, O.Z., Saud, A. 2011. Effect of drought on different physiological characters and yield component in different syrian durum wheat varieties. Journal of Agricultural Science, 3, 127-133.

[3] Viscardi, S., Ventorino, V., Duran, P., Maggio, A., De Pascale, S., Mora, M.L., Pepe, O. 2016. Assessment of plant growth promoting activities and abiotic stress tolerance of Azotobacter chroococcum strains for a potential use in sustainable agriculture. Journal of Soil Science and Plant Nutrition, 16, 848-863.

[4] Tian, F.P., Zhi, N.Z., Xiao, F.C., Lei, Sun, Xue, H.W., Gao, L.W. 2016. Effects of biotic and abiotic factors on soil organic carbon in semi-arid grassland. Journal of Soil Science and Plant Nutrition, 16, 1087-1096.

[5] Ashraf, M., Mehmood, S. 1990. Response of four Brassica species to drought stress. Environmental and Experimental Botany, 30, 93-100.

[6] Fernandes, G.C.J. 1992. Effective selection criteria for assessing plant stress tolerance. In proceeding of the Int. Symp. Adaptation of Vegetables and other Food Crop in Temperature and Water Stress., 257-270.

[7] Eneas, F.J., Neto, O.B.O., Prisco, J.T., Filho, E.G., Nogueira, C.M. 1995. Effects of salinity *in vivo* and *in vitro* on cotyledonary galactosidases from *vigna unguiculata* (L.) walp during seed germination and seedling establishment. Brazilian Journal of Plant Physiology, 7, 135-142.

[8] Ghoulam, C., Fares, K. 2001. Effect of salinity on seed germination and early seedling growth of sugar beet *(Beta vulgaris* L.). Seed Science and Technology, 29, 357-364.

[9] Mwale, S.S., Hamusimbi, C., Mwansa, K. 2003. Germination, emergence and growth of sunflower (*Helianthus annuus* L.) in response to osmotic seed priming. Seed Science and Technology, 31, 199-206.

[10] Sardare, M.D., Admane, S.V. 2013. A review on plant without soil-hydroponics. International Journal of Research in Engineering, 2, 299-304.

[11] Purohit, M., Srivastava, S., Srivastava, P.S. 1998. Stress tolerant plants through tissue culture. Plant Tissue Culture and Molecular Biology: Application and Prospects. Narosa Publishing House, New Delhi., 554–578.

[12] Wani, S.H., Sofi, P.A., Gosal, S.S., Singh, N.B. 2010. In vitro screening of rice (*Oryza sativa* L) callus for drought tolerance. Communications in Biometry Crop Sci., 5, 108–115.

[13] Emmerich, W.E., Hardgree, S.P. 1990. Polyethylene glycol solution contact effects on seed germination. Agronomy Journal, 82, 1103-1107.

[13] Emmerich, W.E., Hardgree, S.P. 1990. Polyethylene glycol solution contact effects on seed germination. Agronomy Journal, 82, 1103-1107.

[14] Hossain, M.I., Khatun, A., Talukder, M.S.A., Dewan, M.M.R., Uddin, M.S. 2010. Effect of drought on physiology and yield contributing characters of sunflower. Bangladesh Journal of Agricultural Research, 35, 113-124.

[15] Husen, A., Iqbal, M. 2014. Growth, water status, and leaf characteristics of *Brassica carinata* under drought and rehydration conditions. Brazilian Journal of Botany, 37, 217-227.

[16] Gopal, J., Iwama, K. 2007. *In vitro* screening of potato against water-stress mediated through sorbitol and polyethylene glycol. Plant Cell Reports, 26, 693-700.

[17] Tumdam, J.S., Patail, S.R., Uke, A.P., Gaikwad, S.B., Puttawar, M.R., Bansod, S.C. 2012. Effect of in vitro drought stress on brassica species. International Journal of Soil Science, 22, 93-99.

[18] Tsago, Y., Andargie, M., Takele, A. 2013. *In vitro* screening for drought tolerance in different sorghum (*Sorghum bicolor* L. Moench) varieties. Journal of Stress Physiology & Biochemistry, 9, 72-83.

[19] Hadi, F., Ayaz, M., Ali, S., Shafiq, M., Ullah, R., Jan, A.U. 2014. Comparative effect of polyethylene glycol and mannitol induced drought on growth (*in vitro*) of canola (*Brassica napus*), cauliflower (*Brassica oleracea*) and tomato (*Lycopersicon esculentum*) seedlings. International Journal of Biosciences, 4, 34-41.

[20] Moradshahi, A., Eskandari, B.S., Kholdebarin, B. 2004. Some physiological responses of canola (*Brassica napus* L.) to water deficit stress under laboratory conditions. Iranian Journal of Science and Technology, 28.

[21] Plaside, R., Christian, C.S., Rony, S. 2012. Development of *in vitro* technique to screen for drought tolerant banana varieties by sorbitol induced osmotic stress. African Journal of Plant Science, 6, 416-425.

[22] Chandler, S.F., Thorpe, T.A. 1987. Characterization of growth, water relations and proline accumulation in sodium sulfate tolerant callus of *Brassica napus* L. cv Wastar (Canola). Plant Physiol., 84, 106-111.

[23] Nosrati, S., Zanjan, M.J., Asli, D.E. 2014. Fluctuations of proline concentration and soluble sugars content affected drought stress in canola (*Brassica napus* L.) seedlings. Journal of Applied Sciences, 9, 497-502.

[24] Wyn Jones, R.G., Storey, R. 1978. Salt stress and comparative physiology in the Graminae IV, comparison of salt stress in spartina × townsendii and three barley cultivars. Australian journal of plant physiology, 5, 839-850.

[25] Ghuge, S.A., Rai, A.N., Suprasanna, P. 2010. Comparative effects of NaCl, PEG and Mannitol Iso-osmotic stress on solute accumulation and antioxidant enzyme system in potato (Solanum tuberosum L.). Plant Stress., 4, 50-55