Advances in Environmental Biology, 9(2) January 2015, Pages: 135-142

AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/

Comparison of Drought Tolerance of Four Varieties of *Brassica Napus* Under *in Vitro* Culture

[1]Fatemeh Adabavazeh and [2]Roya Razavizadeh

[1]*MSc student, Department of Biology, Payam Noor University, Isfahan, Iran*
[2]*Assistant Professor, Department of Biology, Payame Noor University, Tehran, Iran*

ARTICLE INFO

Article history:
Received 12 October 2014
Received in revised form 26 December 2014
Accepted 1 January 2015
Available online 10 February 2015

Keywords:
Brassica napus, drought stress, in vitro culture

ABSTRACT

Drought is one of the most important environmental factors that are known as limiting factor of plants growth in many parts of the world. Identification of drought tolerant cultivars is the most effective method for increasing yield. In this study, the seeds of four varieties of *Brassica napus* were sterilized and then were cultured on MS medium supplemented with mannitol(at percentages of 0, 2, 4, 6 and 8). After 4 weeks, morphological parameters, contents of photosynthetic pigments (chlorophylls a, b and carotenoid), anthocyanin, flavonoid, proline content and soluble sugar were measured in treated plants. Results showed drought stress increases soluble sugar, anthocyanin, flavonoid, proline accumulation and decreases growth parameters, photosynthesis pigments in four varieties of treated plants. Increased Prolin and soluble sugars as osmotic regulators and increase in flavonoids in scavenging of hydrogene peroxide and other species of active oxygen could consider as a defensive reaction of plants against drought stress Elite was the most tolerant cultivar to drought stress.This variety had the highest level of proline, pigments contents, shoot and weight among four varieties of *Brassica napus* under stress condition

To Cite This Article: Fatemeh Adabavazeh and Roya Razavizadeh., Comparison of Drought Tolerance of Four Varieties of Brassica Napus Under in Vitro Culture. ***Adv. Environ. Biol., 9(2),*** *135-142, 2015*

INTRODUCTION

Rapeseed (*Brassica napus* L.) is an annual herbaceous plant. This plant as a dought-tolerent one with oil-rich seeds is considered to be grown in Iran with regard to the climatic conditions of this country. The oil produced from this plant is a widely valuable nutrient due to the presence unsaturated fatty acids with no cholesterol [29]. High ability of the plant and its adaptation to the different climatic conditions makes it a significant plant compared to other oil plants such as sunflower and safflower; thus, the plant is expected to be a good source to supply the raw oil demand in Iran [20]. *Brassica napus* is mostly produced under rain-fed agriculture conditions; so, the plant reaction to water stress should be taken into account.

Drought is known as the most serious abiotic factor which leaves detrimental impacts on the plants growth and function and makes adverse changes to the molecular and physiological processes within the plants. Drought is detrimental to the plants because it causes oxidation and producing a high amount of reactive oxygen species such as Anion super oxide, Radical hydroxyl, and Hydrogen peroxide which cause chlorophyll breakdown in chloroplast and disappearance of thylacoid membranes which finally disrupt photosynthesis process. In addition, reactive oxygen decreases carotenoids concentration by oxidizing carotenoids and damaging their cell membrane [26]. In order to react to photosynthetic pigments damage, anthocyanins and flavonoids that act as accessory pigments protect the plant from the stress by removing reactive oxygen species [5].

A plant under stress reserves its needed water supply by a series of mechanisms arisen from plant genotype or plant physiology. An important mechanism that helps a plant to react to drought stress is osmotic adjustment [24]. By this mechanism, osmotic potential of the plant is reduced as a result of the accumulation of proline and soluble carbohydrates.

Mannitol is a type of sugar having six carbon molecules. This sugar which is produced naturally in many plants acts as an antioxidant which protects the plant from drought and saline stress. Since this sugar alcohol is

Corresponding Author: Roya razavizadeh, Assistant Professor, Department of Biology, Payame Noor University, Tehran, Iran
E-mail: razavi.roya@gmail.com

not metabolized by the plants, it is used for plant tissue culture and the determination of drought-tolerant plants [9].

Evaluation of drought tolerance is a major step to improve plants. With regard to that some varieties of *Brassica napus* are grown increasingly in dry regions of Iran, it is important to examine drought-tolerance physiological reactions of different varieties of *Brassica napus* to determine the effective tolerance reactions and select the most tolerant species to be grown in water-stressed regions of Iran. Since it is difficult to examine the plant tolerance to drought by traditional methods, these days more recent methods such as tissue culture are used to examine the plant tolerance to drought and other damaging factors [13]. This study aims at examining the effects of different levels of water stress on morphologic, physiologic, and metabolic parameters of four varieties of *Brassica napus* under *in vitro* conditions.

MATERIALS AND METHODS

The dry mature seeds of *B. napus* cultivars Tassilo, Okapi, Elite, and Zarfam were studied for percentage of germination, growth parameters, soluble sugar, anthocyanin, flavonoid, proline accumulation, in shoots and roots. The seeds were provided by the Oil Seed Cultivation Company, Isfahan-Iran. The mature and sterilized seeds were grown on MS [21] medium containing concentrations of 0 (control), 2, 4, 6 and 8 gr mannitol. All cultures then were kept in the culture room with a 16/8- h light/dark photoperiod at 25 ± 2 ºC and 95% relative humidity for 4 weeks.

Measurement of Percentage of germination and growth parameters:

Germination percentage of seeds was determined after 5 and 10 days after culture. Shoot and root lengths, shoot and root fresh weights were measured as the growth parameters of canola plants in response to drought stress. Shoot and root lengths were measured using a centimeter scale and fresh weights were measured using a digital scale (Kern pls precision balance0.001).

Measurement of chlorophyll and carotenoids:

Measurement of chlorophyll and carotenoids was determined according to Lichtenthaler method. 0.05 g of frozen leaf was homogenized in 10 ml acetone 80%. This solution contains chlorophyll a, b and carotenoids. The absorbance of each sample was read at 646.8, 663.20 and 470 nm. The amount of chlorophylls and carotenoids was expressed as g ml -1 FW.

Measurement of anthocyanin:

Measurement of total anthocyanin was determined according to modified Wagner [34] method using acidified ethanol (Ethanol: HCl 99: 1 v/v). 0.05 g of frozen leaf was homogenized in 2.5 ml acidified ethanol and then kept at 25°C for 24 h in the dark. The extract was centrifuged at 4000 g for 10 min at room temperature. The absorbance of each supernatant was read at 550 nm. The extinction coefficient 33,000 (mol-1 cm-1) was used to calculate the amount of total anthocyanin and it was expressed as µ mol g-1 FW.

Measuring flavonoids:

Flavonoids concentration was measured using a spectrophotometric method following Kirzek *et al*. method (1998). Leaf disks were prepared and ground in mortars filled with acid ethanol (Ethylic alcohol and Acetic acid glacial in 99:1 vol/vol.). The extract was treated for 10 minutes in a warm bath under 80 ˚C after centrifuge. Its absorption spectrum was recorded at wavelengths 270, 300, 330 nm.

Measuring the amount of reducing carbohydrates:

Reducing carbohydrates content was measured by adapting Somogyi-Nelson's method (1952).Approximately 0.05 g of fresh leaves and roots were extracted with 10 ml distilled water. The mixture was boiled in a boiling water bath, cooled and filtered. Then 2 ml of the extract was mixed with 2 ml of alkaline copper tartarate and the reaction mixture was heated for 20 min (Alkaline copper tartarate was prepared by dissolving 4 g anhydrous sodium carbonate, 0.75 g tartaric acid and 0.45 g hydrated cupric sulphate in 80 ml of distilled water and finally made up to 100 ml). Two ml of phosphomolibdate solution was added and the intensity of blue color was measured at 600 nm using spectrophotometer. D-glucose was used as standard. The reducing sugar content was expressed as mg/g FW.

Measurement of proline:

Proline content was estimated using ninhydrin reaction (Bates *et al*. 1973). A small portion (0.5 g) of leaves or roots was homogenized with 10 ml of 3% (w/v) sulphosalicylic acid, and passed through Whatman filter paper no. 2. Then ninhydrin reagent (2 ml) (Sigma) and glacial acetic acid (2 ml) were added to 2 ml of the filtered extract. The mixture was incubated at 100°C for 1 h, and the reaction was terminated by placing it on

Advances in Environmental Biology, 9(2) January 2015, Pages: 135-142

ice. The reaction mixture was extracted with 4 ml toluene, and absorption of chromophore was measured at 520 nm, against toluene as blank, using spectrophotometer (Shimadzu UV-160, Japan). Proline content was calculated using L-proline (Sigma) as a standard curve.

All experiments were carried out in three replications and mean values ± standard deviation were presented. Data were subjected to ANOVA using the statistical package SPSS and the mean differences were compared by Dunkan test at $p < 0.05$.

Results:

Assessment of ability to absorb water for seed germination and seedling growth under water stress conditions *in vitro* culture by measuring the length of the stem and the root was performed. Statistical calculations done at level 0.05 by SPSS software indicated that drought stress had significant influence on germination rate, shoot and root lengths, shoot and root fresh weights. It was found that the increase of water stress decreases these characteristics in all varieties.

Germination rate:

In this study, it was observed that water potential decrease and drought levels increase significantly affected the germination of *B. napus* seeds. As figure 1 shows germination percentage of canola seeds cultivars Tassilo, Okapi, Elite, and Zarfam decreased significantly due to the addition of mannitol to medium.The maximum seed germination was observed in Tassilo as compared to control plants.

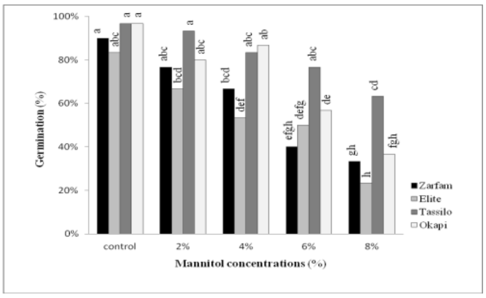

Fig. 1: The effect of mannitol on the germination percentage of *Brassica napus* varieties after 7 days. Values represent the mean of three replicates and dissimilar letters are significantly different according to Duncan's test (P ≤ 005).

Growth parameters:

Application of the concentrations of mannitol to canola plants variously influenced their growth parameters (shoot and root length, fresh weight of shoots and roots) as compared to control plants**.** The present study, all growth parameters such as length and fresh weight of shoot and root decreased with increase in drought levels. Drought treatments caused significant reduction in shoot length and fresh weight of shoot (Figure 2 a and b). The interactions between variety and drought treatment were not significant. In addition, reducing of root length, fresh weight of root were presented under water stress in the presence of mannitol treatment (Figure 3 a and b). Among four varieties, Okapi showed the highest growth rate and Zarfam showed the lowest growth rate at treatment level 8%.

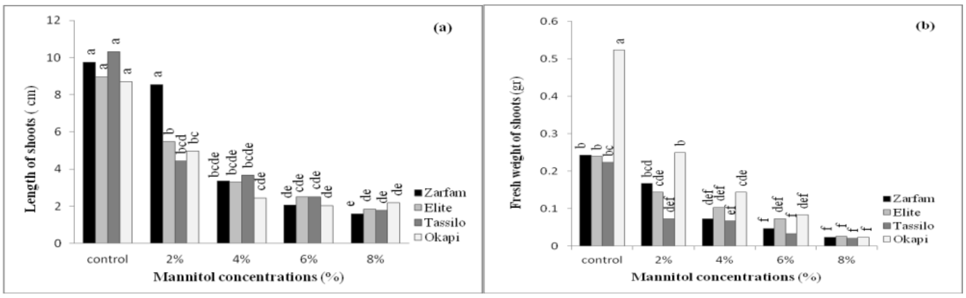

Fig. 2: The effect of mannitol on the shoot length (a) and shoot fresh weight (b) of *Brassica napus* varieties. Values represent the mean of three replicates and dissimilar letters are significantly different according to Duncan's test (P ≤ 005).

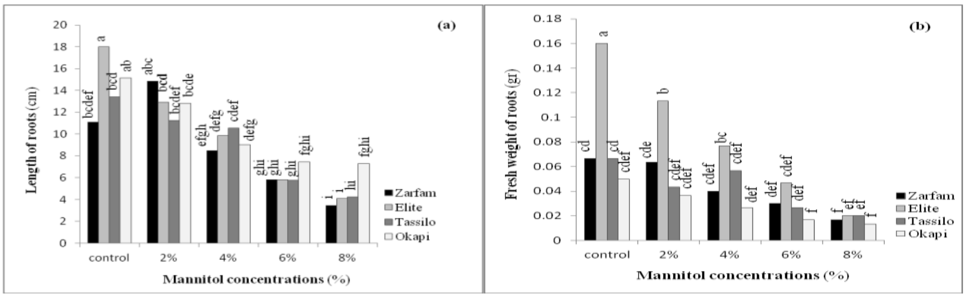

Fig. 3: The effect of mannitol on the root length (a) root fresh weight (b) of *Brassica napus* varieties. Values represent the mean of three replicates and dissimilar letters are significantly different according to Duncan's test (P ≤ 005).

Chlorophyll a, b, total chlorophyll content, and carotenoids:

The varieties under drought treatment showed significant losses in chlorophyll a and b, total chlorophyll and carotenoids content compared to control plants. Accorrding to the results obtained from the increase of drought stress levels, a statistically significant difference was observed in chlorophyll a and b and total chlorophyll content among varieties under different levels of drought treatment. In addition different osmotic potential treatment affected each variety differently (Figure 4). Among varieties, Elite and Zarfam showed the highest and lowest photosynthetic pigment concentration respectively.

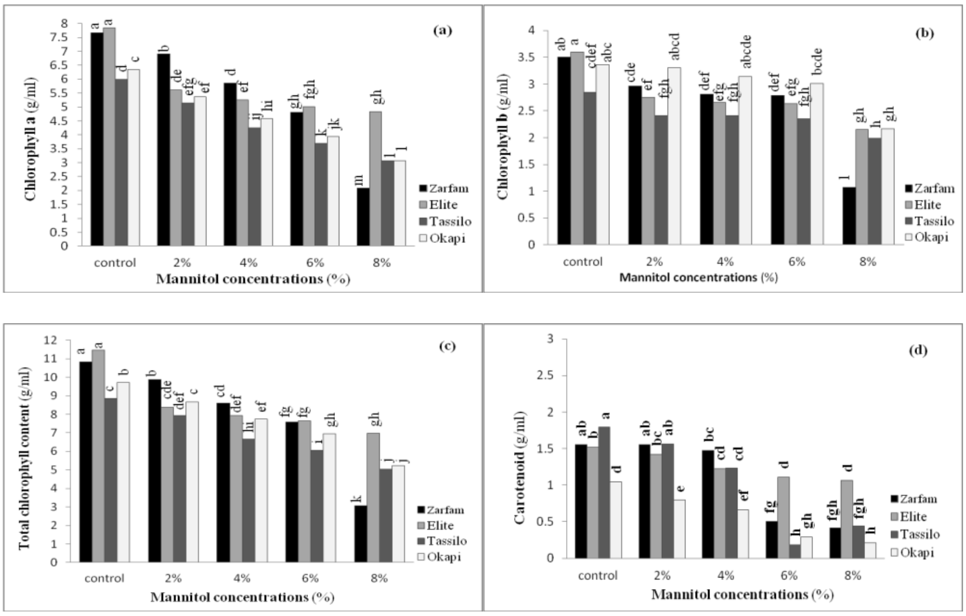

Fig. 4: The effect of mannitol on the amount of Chlorophyll a (a), chlorophyll b (b), total chlorophyll (c), and Carotenoids (d) of *Brassica napus* varieties. Values represent the mean of three replicates and dissimilar letters are significantly different according to Duncan's test (P ≤ 005).

Flavonoids:

All four varieties showed an increase in flavonoids content under the increase of drought stress. According to the results of the statistical calculations, a significant increase was observed in flavonoid content treated plants compared to untreated plants at different drought stress levels. The highest Flavonoid content was observed in Okapi at 8% drought stress level (Figure 5a).

Anthocyanins:

Higher concentration of mannitol induced a significant increase in anthocyanin content in comparison to the control. The highest anthocyanin content was observed in Tassilo at 8% stress level (Figure 5b).

Proline content:

It was statistically observed that drought stress had a significant influence on proline content of shoots in all varieties. The interactions between variety and drought treatment were significant. The result of present study has indicated that drought stress was effective in increasing of proline content of shoots in all varieties and significantly increased it than control (Figure 6a). Proline content was the highest in Elite.

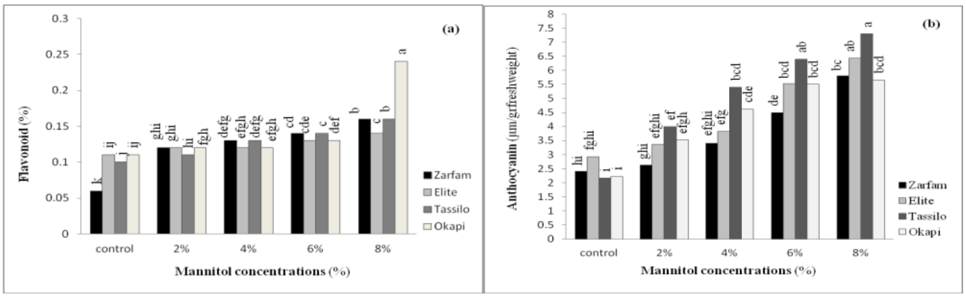

Fig. 5: The effect of mannitol on flavonoids content (a) and anthocyanins content (b) of *Brassica napus* varieties. Values represent the mean of three replicates and dissimilar letters are significantly different according to Duncan's test (P ≤ 005).

Soluble sugars:

Drought stress had a significant influence on soluble sugar concentration in shoots. The present results demonstrated that drought sress was significantly improved the reducing sugars production and accumulation in shoots of four varieties of *B. napus*). Also four varieties showed the highest value of soluble sugars content in shoots at 8% stress level (Figure 6b). The interactions between variety and drought treatment were also significant. Zarfam showed the highest sugar content as compared to other varieties.

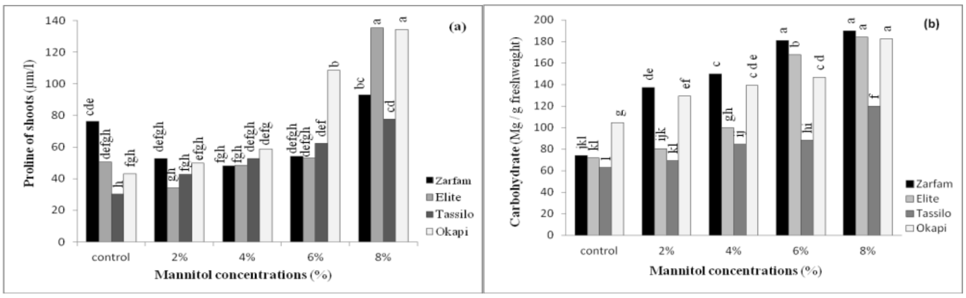

Fig. 6: The effect of mannitol on proline content (a) reducing sugars of shoots (b) of *Brassica napus* varieties. Values represent the mean of three replicates and dissimilar letters are significantly different according to Duncan's test (P ≤ 005).

Discussion:

Drought stress is the most important biotic factor disrupting plants germination and growth. Loss of water potential is a common result of drought stress. High negative water potential inhibits seed to absorb water and blocks germination processes in the plant.

According to the observations in this experiment, germination rate decreased in four varieties under drought stress. But, the highest germination rate was observed in Tassilo. In this regard, it is noted that seeds have to absorb high enough water to conduct germination process; but soluble substances such as Poly-Ethylene Glycol that exist in medium reduce water absorbance rate in the plants and inhibit germination [33]. The decrease of germination rate is resulted mainly from the change in transporting seed nutrients and protein synthesis under stress conditions.

Also, a decrease in the length of stem and root of the varieties was observed. Such a decrease is common in the plants under stress and is a result of a change in the plant metabolism. Studies show that the plants change their morphologic, physiologic, and anatomic characteristics to increase their tolerance to the drought stress. In this study Okapi and Elite showed the highest growth value at 8% manitol. Whereas Okapi showed the highest growth value, Zarfam showed the lowest length of stem and root. In a study on the effect of water stress resulted by Poly-ethylene glycol on the growth and germination of Millet and Sorghum seeds observed that increase in stress level from -0.4 Mpa upward results in a significant decrease in the length of the roots of the varieties under study. In another study on the effects of water stress resulted by Poly-ethylene glycol on the growth and germination rate of chickpea cultivar (*Cicer arietinum*), It was observed that loss of water potential results in a significant decrease in the length of the stem and root of cultivar under study [15].

Usually drought stress increase results in a decrease in fresh weight. In this study, it was also observed that drought stress resulted in a significant decrease in fresh weight of roots and shoots in four varieties. According to the observations of this experiment, Elite showed the highest fresh weight under 8% treatments. It seems that fresh weight reduction in the plants under stress treatment may be a result of inhibition of cell growth as a result of turgor pressure reduction [25]. In another study observed that the fresh weight of wheat's cultivar may be caused by reduction of nutrients movements in the seeds and plants weakness to transport nutrients from seeds to xylem [31].

Drought stress affected photosynthetic pigments significantly and decreased all photosynthetic pigments. Decreases in photosynthetic pigments were due to instability of protein complexes and destruction of chlorophyll by increased activity of chlorophyll degrading enzymes and chlorophyllase under stress condition. The reduction of chlorophyll due to stress is related to the increase of production of Reactive oxygen species (ROS) in the cell. These radicals cause peroxidation, disintegration and reduction of chlorophyll content in plants under stressful conditions. It seems that osmotic shock resulted by mannitol, increases some growth regulator substances such as ethylene and abscisic acid. This process causes to stimulate chlorophyllase and degrades chlorophyll. When a chlorophyll molecule breaks down, chlorophyllides will be produced and after opening prophyrin rings are moved to vacuole [26]. Other studies in the field also observed that the reduction of water potential in the leaves of wheats caused in an increase in chlorophyllase activities [19]. In addition, drought stress disrupts the activity of enzymes preventing reactive oxygen species activity and increases lipid peroxidation and thus damages to the cell membrane and pigments [3]. Some of these results are in agreement with the earlier findings in *Salvia Leriifolia* [12]. Similar results were also reported in *Phragmites australis*. Under drought stress Carotenoids are reduced and are not able to function as accessory pigments. In the present study, a significant decrease was observed in Carotenoids content under stress. The highest value for Carotenoids content was shown by Elite at 8% stress level. Carotenoids cause the protection of photosynthesis system against extra photons and oxidative stress such as reaction to chlorophyll to prevent the formation of active radicals of oxygen. In fact, carotenoids as a protective system against induced oxidative stress are disintegrated and destroyed. The photochemical suppression of induced chlorophylls by carotenoids results in the disruption of carotenoid structure and finally reduction in their amount.

Many observations indicate that under stress, the production of some secondary metabolites increase in the plant. Flavonoids are a type of secondary metabolites and polyphonic compounds of plants. These components are derivatives of phenyl propanoid. According to the observations of this experiment, flavonoids concentration increased in four varieties under stress. The highest value was observed in Okapi at 8% stress level. The results obtained from this study confirm the results of studies on *Linum usitatissimum* L. which showed an increase in flavonoids content under stress. This increase may result from high volume of reactive oxygen species such as anion super oxide, radical hydroxyl, and hydrogen peroxide. Under such conditions, flavonoids identify the number and the situation of phenolic OH groups and start erasing operation [18]. In addition, other similar results were reported in *Stellaria Longips [28]*. According to the results of this study, flavonoids can scavenge reactive oxygen species.

Anthocyanins also function as accessory pigments which protect the plant from water stress [5]. Anthocyanin as a group of soluble flavonoids is synthesized in the pathway of flavonoid biosynthesis at the end point [16]. By creating oxidative stress in plant, antioxidant genes expression [32] and induction of phenyl propanoid pathway especially Flavonoid biosynthesis are increased [8]. Under stress condition, anthocyanins content increased in four varieties. The highest value for anthocyanin concentration was observed in Tassilo followed by Elite. Similar result was reported in *Begonia semperflorens* [36]. According to the results, the increase occurs because anthocyanins function as Light protection by erasing reactive oxygen species during oxidation stress.

To retain ion balance and osmotic regulation in vacuoles and cytoplasm, the plants accumulate low molecular weight compounds such as proline, glycine, betaine and sugars like glucose and fructose that are collectively called osmolite [21]. Under stress, prolin content increased in all four varieties. The highest value was observed in Elite followed by Okapi at 8% stress level. Proline is an amino acid. Proline increase is the most common and immediate response of plants to the stress. Proline accumulation may occur because it functions as an osmolite in osmotic adjustment and protects many proteins and cytoplasmic enzymes [6]. Other studies also reported similar results about increase in proline concentration. It was observed that proline content in *Sesuvium portulacastrum* cultivar increased 3 times compared to that of control group. The study suggests that the increase may occur because over expression of ornithine omega-aminotransferases enzyme in proline biosynthesis pathway and inhibition of proline dyhydrogenase activity. In a study on the effect of water and salt stress on *Triticum durum* cultivar observed that these stresses result in the increase of amino acids concentration especially proline amino acid [17]. Water stress may result increase in proline content by increasing enzymes biosynthesizing proline and reducing enzymes inhibiting proline [35].

Drought directly or indirectly cause the accumulation of reactive oxygen species lead to the accumulation of soluble sugars that act as an adaptive mechanism to stress condition [32]. It seems that soluble sugars play an important role in relation with reactive oxygen species. Sugars are also needed for performing anti-oxidative processes like pentose phosphate pathway [1] and carotenoid biosynthesis. Soluble sugars are accumulated in response to abiotic stresses and have an impact on osmotic adjustment, protecting cell membranes, and erasing oxygen free radicals. Current paper shows that sugar concentration increases under stress. Higher values in this regard were observed in Zarfam and Elite. Researchers attribute soluble sugar content increase to factors such as the increase of non-soluble carbohydrates break down, increase of soluble sugar levels, osmotic substances synthesis via non-photosynthetic paths, growth inhibition, reduction of substances transportation speed, and the increase of Sucrose synthesis rate due to the activation of sucrose phosphate synthase [22]. Results of another study on chickpeas were also similar to the results of our study [27].

Conclusion:

The results of this study showed that drought stress had adverse effects on morphological and physiological parameters of *Brassica napus* varieties. It was observed that canola plants are somewhat resistant to water stress. In general it can be concluded that *Brassica napus* cultivars show effective responses to stress to be protected from damage. These responses depend on the severity of the stress and plant variety. According to the different characteristics studied in this study can be said among four varieties, Elite was the most tolerant variety and Zarfam was the most sensitive one.

REFERENCES

[1] Barra, L., N. Pica, K. Couffi, G. Walker, C. Blanko and A. Trautwetter, 2003. Glocose-6-phosfate dehydrogenase is required for sucrose and trehalose to be efficient osmoprotectans in *Sinorhizobium meliloty*. FEMS Microbiology Letters, 229: 183-188.

[2] Bates, L.S., R.P. Waldern and I.D. Teare, 1973. Rapid determination of free proline for water stress studies. Journal: Plant and Soil, 39(1): 205-207.

[3] Bowler, C., M. Van Montagu and D. Inze, 1992. Superoxide dismutaze and stress tolerance. Plant Physiology, 43: 83-116.

[4] Boydak, M., H. Dirik, F. Tilki and M. Calikoglu, 2003. Effects of water stress on germination in six provenances of *Pinus brutia* seeds from diff erent bioclimatic zones in Turkey.Turkish Journal of Agriculture and Forestry, 27: 91-97.

[5] Chalker-Scott, L., 2002. Do anthocyanins function as osmoregulators in leaf tissues? Advances in Botanical Research, 37: 103-106.

[6] Delauney, A.J. and D.P. Verma, 1993. Proline biosynthesis and osmoregulation in plants.The Plant Journal, 4: 215-223.

[7] Dodd, G.L. and L.A. Donovan, 1999.Water potential and ionic effects on germination and seedling growth of two cold desert shrubs.The American Journal of Botany, 86: 1146-1153.

[8] Green, R. and R. Fluhr, 1995. UV-B induced PR-1 accumulation is mediated by active oxygen species. Plant Cell, 7: 203-212.

[9] Hassanein, A. and Dorion, 2006b. High efficiency colony formation and whole plant regeneration from mesophyll protoplasts of *Pelargonium X hortorum 'panache sud'*.The Journal of Horticultural Science and Biotechnology, 81(4): 714-720.

[10] Khalsro sh. and M. Qalykhany, 1386. Effect of salinity and water stress on seed germination ofSorghumand Millet. Research and Construction, 153-163: 77.

[11] Krizek, D.T., S.J. Britz and R.M. Mirecki, 1998. Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of *cv.* new red fire lettuce.Physiologia Plantarum, 103: 1-7.

[12] Lahoti Mehrdad, 2011. Comparison of the effects of drought stress, proline of *Salvia leriifolia* in the soil and *in vitro* culture.Journal of Biological Sciences, Zanjan University, 4: 105-115.

[13] Larkin, P.J. and W.R. Scowcroft, 1981. Somaclonal variation a novel source variability from cell culture for plant improvement.Theoretical and Applied Genetics, 60: 197-214.

[14] Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology, 148: 350-382.

[15] Macar, T.K., O. Turan and Y. Ekmekci, 2009. Effects of water deficit induced by PEG and NaCl on chickpea (*Cicer arietinum* L.) cultivars and lines at early seedling stages. Gazi University Journal of Science, 22: 5-14.

[16] Mars, K.A., M.R. Alfenito, A.M. Loyd and V.A. Valbot, 1995. Glutathione s-transferase involved in vacuolar transfer encoded by the maize gene bronze-2. Nuture, 375: 397-400.

[17] Mattioni, C., N.G. Lacerenza, A. Troccoli, A.M. De Leonardis and N. Di Fonzo, 1997. Water and salt stress-induced alteration in proline metabolism of *Triticum durum* seedlings. Physiologia Plantarum. 101 (4); 787-792.

[18] Middleton Jr, E., C. Kandaswami T.C. Theoharides 2000. The Effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer.Pharmacological Reviews, 52(4): 673-751.

Advances in Environmental Biology, 9(2) January 2015, Pages: 135-142

[19] Mihalovic, N., M. Lazarevic, Z. Dzeletoric, M. Vuckoric and M. Durde Vic, 1997. Chlorophyllas activity in Wheat leaves during drought and its dependence on the nitrogen ion from applied. Plant Science, 129: 141-146.
[20] Motamedi, B. and F. Javidfar, 1380. Planting and harvesting Canola, 15-13: 46.
[21] Murali, N.S. and A.H. Teramura, 1986. Effectiveness of UV-B radiation on the growth and physiology of field grown soya bean modified by water stress. Photochemical Photobiology, 44: 215-219.
[22] Oliviera-Neto, C.F., A.K. Silva-Lobato, M.C. Goncalves-Vidigal and R.C.L. Costa, 2009. Carbon compounds and chlorophyll contents in sorghum submitted to water deficit during three growth stages. Science and Technology, 7: 588-593.
[23] Oraki, H., F. Parhizkar khajani and M. Aghaalikhana, 2012. Effect of water deficit stress on proline contents, soluble sugars, chlorophyll and grain yield of sunflower (*Helianthus annuus* L.) hybrids. African Journal of Biotechnology, 25: 164-168.
[24] Pagter, M., C. Bragato and H. Brix, 2005.Tolerance and physiological responses of Phragmites australis to water deficit. Aquatic Botany, 81: 285-299.
[25] Rane, J. and M. Maheshwari, 2001. Effect of pre-anthesis water stress on growth, photosynthesis and yield of six wheat cultivars differing in drought tolerance. Indian Journal of Plant Physiology, 6: 53-60.
[26] Sairam, R.K., P.S. Deshmukh and D.C. Saxna, 1998. Role of antioxidant systems in Wheat genotype tolerance to water stress. Biologia Plantrum, 41(3): 387- 394.
[27] Schubert, S., R. Serraj and P.E. Balzer, 1995. Effect of drought stress on growth, sugar concentrations and amino acid accumulation in N-2-fixiy alfalfa Journal of plant physiology, 146(4): 541-546.
[28] Seyoum, A., K. Asres and F.K. El-Fiky, 2006. Structure radical scavenging activity relationships of flavonoid. Phytochemistry, 67(18): 2058-2070.
[29] Shahidi, A. and K. Forozanm, 1376. Canola.Joint Stock Company of oilseeds cultivation.
[30] Slama, I., D. Messedi, T. Ghanaya, A. Savoure and C. Abdelly, 2005. Effects of water deficit on growth and proline metabolism in *Sesuvium portulacastrum*.Environmental and Experimental Botany, 56: 231-238.
[31] Soltani, A., and S. Galeshi, 2002. Importance of rapid canopy closure for wheat production in a temperate subhumid environment: experimentation and simulation. Field Crops Resesearch, 77: 17-30.
[32] Somogyi-Nelson, M., 1952. Notes on sugar determination. Journal of Biological Chemistry, 195:19-29.
[33] Tobe, K., L. Zhang, G.Y. Qiu and H. Shimizu, 2001. Characteristics of seed germination in five non-halophytic Chinese desert shrub species. Journal of Arid Environments, 47: 191-201.
[34] Wagner G.J., 1979. Content and vacuole/extravacuole distribution of neutral sugars, free amino acids, and anthocyanins in protoplast.Plant Physiology, 64: 88-93.
[35] Zhang, J., H.T. Nguyen and A. Blum, 1999. Genetic analysis of osmotic adjustment in crop plants.Journal of Experimental Botany, 50(332): 291- 302.
[36] Zhang,. K.M., H.J. Yu, K. Shi, Y.H. Zhou J.Q. Yu and X.J. Xia, 2010. Photoprotective roles of anthocyanins in *Begonia semperflorens*. Plant Science, 179(3): 202-208.