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ORIGINAL ARTICLE

Effects of Water Stress and Nitrogen on Changes of Some Amino Acids and Pigments in Canola

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ABSTRACT
To evaluate the responses of canola seedlings to different levels of drought stress and nitrogen at different growth stages, an experiment was conducted as a factorial in a completely randomized design with three replications at the experimental greenhouse of Mohaghegh Ardabili University in 2013. Treatments included three levels of drought stress (30%, 50% and 70% of FC) and five levels of nitrogen (Control based on soil test results, 25% less than the recommended level, 50% less than the recommended level, 25% more than the recommended level and 50% more than the recommended level). The recommended level of nitrogen, based on soil test results, was 0.09 g of nitrogen per kg of soil.ANOVA results showed that water stress × nitrogen significantly affected the total soluble protein content, lysine and proline amino acids content, chlorophyll a/b ratio and carotenoids content in all stages of sampling (4-6, 6-8 and 8-10 leaf stages). The total soluble protein content increased with the advancement of plant growth. The highest total soluble protein content (28.63 mg/g FW) was observed at 8-10 leaf stage under severe water stress conditions (30% of FC) and the application of 0.09 g N per kg of soil (control). Changes in lysine and methionine amino acids were different over the growth period, so that the lysine content was decreased at 8-10 leaf stage compared to that of 4-6 leaf stage while the methionine was accumulated over the same period of time.
Keywords: *Stress, Drought, Lysine, Methionine, Soluble Sugars.*

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INTRODUCTION

Abiotic stresses lead to the production of high levels of Reactive Oxygen Species (ROS) in plants which cause damage to proteins, lipids, carbohydrates and DNA. Plants use both enzymatic and non-enzymatic defense mechanisms to neutralize the harmful effects of ROS. Among the non-enzymatic defense mechanisms are the changes made in amino acids and carotenoids contents [39]. Plants responses to stress are complex and depend on some factors associated with morphological, biochemical and physiological processes [35]. Organic solutes such as sugars, organic acids, polyols and nitrogen-containing compounds such as amino acids, amides, proteins and ammonium compounds help to maintain osmotic adjustment [18]. Synthesis and accumulation of low molecular weight metabolites, which are being known as compatible solutes, is considered as an osmotic adjustment mechanism in plants and its major role is to increase the ability of cells to retain water without affecting their normal metabolism. The accumulation of non-toxic compatible solutes in higher concentrations, helps to maintain the turgor pressure or to protect the macromolecular structures against the effects of reduced water activity [20].

Proteins are sensitive to the chemical damages caused by ROS andstudies suggest that amino acids react with ROS. Methionine is highly sensitive to oxidative stress and its oxidation into methionine sulfoxide (MetSO) leads to a change in its activity and formation of many proteins [12].Many studies have shown that hydrophilic amino acids act as antioxidants by increasing their solubility, therefore better act in

response to free radicals [23, 26, 36, 37]. Some amino acids such as histidine, tyrosine, methionine, cysteine and tryptophan have been accepted as antioxidants despite their occasional oxidative behavior [10]. Several mechanisms have evolved in plants in order to get rid of excess energy in photosynthetic membranes. Carotenoids, zeaxanthin and tocopherols play an important protective role in all photosynthetic organisms by transforming the excitation energy into heat or scavenging the ROS [41].Various environmental stresses decrease the photosynthetic pigments content [6].Drought stress not only damages the photosynthetic pigments, but also destroys the thylakoid membranes [3, 22, 24]. Therefore, a decrease in photosynthetic capacity of plants subjected to drought stress is not unexpected [6].

Photosynthetic pigments played an important role in radiation absorption and dissipation of excess energy and the chlorophyll a and b contents were changed under water deficit conditions. An increase in chlorophyll and carotenoids contents under water deficit conditions could be due to a decrease in leaf area or a defensive response against the detrimental effects of water stress [16]. Chlorophyll content in two cultivars of *chrysanthemum* sp. was respectively decreased by 49.7 and 25.4 percent under drought stress (for 7 days) but it was later increased after re-watering them by 92.8 and 68.4 percent, respectively [44].The chlorophyll a/b ratio gradually increased in mutant *Arabidopsis*plants after 24h of drought stress. It was also increased significantly during the drought stress period in wild *Arabidopsis*plants except for a slight decrease at 48h after stress. The chlorophyll a/b ratio in wild *Arabidopsis*plants was 1.2 and 1.4 times higher than that of mutant ones after 48 and 72h of drought stress, respectively [33].

Plants that were subjected to water stress, demonstrated a higher adaptability with higher levels of nitrogen application. Drought tolerance could therefore be attributed to an increase in nitrogen application which prevented damages to cell membrane and enhanced the osmotic adjustment [38]. The photosynthesis rate, chlorophyll and soluble proteins contents are different in plants under various moisture and nutritional conditions. Nitrogen is a component of chlorophyll and enzymes. More than half of the enzymes are in form of soluble proteins and many are involved in photosynthesis [1, 2, 15].An increased application of nitrogen enhanced the photosynthetic activities through an increase in soluble proteins content [50]. Soluble proteins content was also significantly correlated to chlorophyll content and the net photosynthetic rate [48, 50]. The optimal nitrogen nutrition is the basis of plant's growth and productivity and is also essential for the biosynthesis of amino acids, proteins and enzymes [42]. Nitrogen deficiency could also be considered as an abiotic stress which decreases the yield [49].

Based on what was mentioned above, this study was conducted to evaluate the effects of drought stress and different levels of nitrogen application on responses of and changes in soluble proteins content, amino acids and pigments in canola seedlings.

MATERIALS AND METHODS

Lysine and methionine content assay

Leaf samples were well grinded in a mortar containing 0.1% HCl. The resulted solution was then mixed with 50% glycerol, phosphate buffer and ninhydrin in order to extract lysine. It was then placed in the boiling water (100°C). The absorption was read at 570 nm. Methionine was also extracted by adding NaOH (5N), glycine dihydrate (50%), sodium nitroferricyanide dihydrate (0.1%)and HCl (1:1) to the resulted solution mentioned above and its absorption was read at 510 nm [28].

Photosynthetic pigments assay

Fresh leaf tissue was used for the measurement of chlorophyll. 0.2 g of leaf tissue was gradually grinded using 80% acetone in order to extract chlorophyll into the acetone solution. The final volume of the solution was then brought up to 20 ml using 80% acetone. The resulted solution was centrifuged at 400 rpm for 10 min and the optical absorption of the supernatant was then read at both 645 and 663 nm using a spectrophotometer. Chlorophyll and carotenoids contents were obtained according to the following equations [5].

Chlorophyll a= (19.3×A663-0.86×A645)V/100W
Chlorophyll b= (19.3×A645-3.6×A663)V/100W
Chlorophyll t= chlorophyll a + chlorophyll b
Carotenoids= (1000a470-1.82Ca-85.02Cb)/198
Chlorophyll a/b= Chlorophyll a/ Chlorophyll b

Total Soluble Sugar assay

To measure the amount of carbohydrates, an alcoholic extract was first prepared from the leaves. In order to that, 0.5 g of leaf tissue kept at -70°C in refrigerator was first completely homogenized using a porcelain mortar. Then 5 ml of 95% ethanol was added to it and it was transferred into a capped test tube and vortexed for 30 seconds. The supernatant was separated and transferred to another tube and then 70% ethanol was added to the remaining solid part twice, 5 ml each time, and washed out completely.

The supernatant was ultimately transferred to a test tube and 15 ml of the extract was obtained. The resulted extract was then centrifuged at 3500 rpm for 15 min. The supernatant solution was then used to measure the soluble sugars according to the method proposed by Omokolo et al [34]. The absorbance was then measured using a spectrophotometer at a wavelength of 625 nm.
Statistical analysis was performed using SAS software. Mean comparisons was also performed using Duncan's multiple range test at P≤0.05.

RESULTSAND DISCUSSION

Total protein

ANOVA results showed that water stress × nitrogen significantly affected all the evaluated traits at all sampling stages (4-6, 6-8 and 8-10 leaf stage) in this study (data not shown). The highest total protein content was observed at 4-6 leaf stage under severe water stress conditions (30% of FC) and the application of N at the recommended rate (N3) (Figure 1a). The lowest total protein content (2.43 mg/g FW) however, was achieved under mild water stress conditions (50% of FC) and the application of N1 (0.04 g N per kg of soil) (Figure 1a). Unlike the 4-6 leaf stage, the highest total protein content (19.33 mg/g FW) at 6-8 leaf stage was observed under mild water stress conditions (50% of FC) and the application of N at 50% more than the recommended rate (Figure 1b). The accumulation of total protein content increased with the advancement of plant growth (Figure 1c).The significant interaction between water stress and nitrogen may indicate that the application of different rates of N under various moisture conditions would have different effects on total protein content. An increased application of N under both mild (50% of FC) and severe water stress conditions (30% of FC) during the 6-8 leaf stage, increased the total protein content but decreased it under favorable moisture conditions (FC of 70%). Generally, the accumulation of total protein was increased with the advancement of plant growth due to canola seedlings response to increased rates of nitrogen application under both favorable moisture and mild water stress conditions. Results proved that nitrogen plays its protective role against the adverse effects of water stress partly based on its nutritional function. Zhou *et al.* (2011) also suggested that an increased rate of N application had a positive correlation with an increase in total soluble protein content [50]. Changes in protein content in response to abiotic stresses has a complex mechanism which in most cases depends on the plant species and is sometimes independent of the genotype [19]. An increase in protein content under stress conditions is mainly due to a decrease in starch to protein ratio rather than an absolute increase of protein content [30].

Lysine

Mean comparison results for water stress × nitrogen show that any increase in N application rate up to N4 under optimal moisture conditions increased the lysine content at all sampling stages (Figures 2a, 2b and 2c). Unlike the 4-6 leaf stage in which the highest lysine content was achieved under mild water stress conditions in most of the N application treatments (Figure 2a), the accumulation of lysine decreased under all moisture conditions with the advancement of plant growth (up to the 8-10 leaf stage) (Figures 2a and 2c). Increased N application rates up to 0.11g per kg of soil (N4), increased the lysine content under favorable moisture conditions in all three stages of sampling. An increase in water stress severity during the 6-8 leaf stage also resulted in an increase of lysine content compared to the favorable moisture conditions (Figure 2b). The higher lysine contents observed in plants under mild and severe water stress conditions compared to those under favorable moisture conditions which even received greater amounts of N may indicate that water stress had a greater impact than nitrogen on the production of lysine. A decrease in lysine content during the growth is due to the catabolism of this amino acid that leads to the formation of substances which protect the plants against the adverse effects of water stress. Lysine may cause an increased water stress tolerance through its transformation into proline [21],aminobutyric acid [9] and arginine, a potential precursor of polyamines [27]. Therefore, any decrease in lysine content during the growth may indicate its transformation into other substances to raise the level of stress tolerance in plants.

Methionine

Methionine content decreased at 4-6 leaf stage under severe water stress conditions (30% of FC) with increasing rates of N application (Figure 3a). The highest methionine content (0.064) at 6-8 leaf stage was however observed under mild water stress conditions (50% of FC) with the application of 0.13 g N per kg of soil (N5) (Figure 3b). Methionine accumulation was increased exponentially under both mild and severe water stress conditions (50% and 30% of FC, respectively) during the 8-10 leaf stage. With the advancement of plant growth, the potential of higher N application rates to produce methionine was also increased so the application of N more than the recommended rate (N4 and N5 treatments) during the 8-10 leaf stage under mild and severe water stress conditions, increased the methionine content more than the similar conditions during the previous growth stages. Methionine may also enhance the defensive

capability of plants against the water deficit through its transformation into polyamines. The binding of free polyamines to macromolecules protects them against the damages caused by oxidative stress. The free polyamines, however, are primarily involved in maintaining the osmotic and cellular pH balances [29].

Chlorophyll a/b

Mean comparison results showed that there was no significant difference between the treatments for chlorophyll a/b ratio under favorable moisture conditions (FC of 70%) at 4-6 leaf stage. However, the lowest chlorophyll a/b ratio was obtained with the application of N at the recommended rate (N3) under both mild (FC of 50%) and severe water stress conditions (FC of 30%) at the same stage (Figure 4a). Chlorophyll a/b ratio was increased under favorable moisture conditions during the 6-8 leaf stage (Figure 4b) compared to that at 4-6 leaf stage (Figure 4a). Chlorophyll a/b ratio was also lower under favorable moisture conditions during the 8-10 leaf stage compared to that of mild and severe water stress conditions for all the N application treatments (Figure 4c). Mixed results have been reported for chlorophyll a/b ratio. Antolin*et al*. (1995) reported an increase in chlorophyll a/b ratio under severe water stress conditions [4]. However, a decrease in chlorophyll a/b ratio has also been reported in three canola varieties under water stress conditions [7]. Seedlings under mild water stress conditions (50% of FC) in this study showed a positive response to added nitrogen in higher than recommended (control) rates of N application at 4-6 leaf stage through an increase in chlorophyll a/b ratio (Figure 4a) and the same was observed during the 8-10 leaf stage too (Figure 4c). Zhou *et al*. (2011) also reported the positive impact of N on increasing the chlorophyll content [50]. Water stress changes the leaves anatomy and make them smaller but thicker resulting in high concentrations of chlorophyll per unit leaf area so that the photosynthetic rate per unit area may be less affected but it would be reduced in leaves and the whole plant. Estill *et al*. (1991) reported an increased chlorophyll a/b ratio due to changes in photosynthetic systems through a lower ratio of photosystem II/I under water stress conditions [13].

Carotenoids

The highest carotenoids content was observed under favorable moisture conditions (FC of 70%) with the application of 0.13g of N per kg of soil at 4-6 leaf stage (Figure 5a). An increased rate of N application under favorable moisture conditions had a greater impact on carotenoids content increase compared to that of mild and severe water stress conditions during the 4-6 leaf stage (Figure 5a). Plants exposed to mild water stress (50% of FC) reacted positively to an increased rate of N application during the 6-8 leaf stage in terms of carotenoids content (Figure 5b). The lowest carotenoids content, however, was observed under severe water stress conditions with the application of 0.04g of N per kg of soil (N1) at 8-10 leaf stage (Figure 5c).An increased rate of N application resulted in higher carotenoids content under favorable moisture conditions. An increased rate of N application under severe water stress conditions (30% of FC) at 4-6 leaf stage increased the carotenoids content (Figure 5a) but the same did not happen under similar conditions during the 6-8 leaf stage (Figure 5b). Schwanz and Polle (2001) reported that carotenoids content were affected by water deficit and reduced under severe water stress conditions [40]. Higher rates of N application (N4 and N5) also increased the carotenoids content under severe water stress conditions during the 8-10 leaf stage (Figure 5c). Although the carotenoids content was decreased under severe water stress conditions during the 6-8 leaf stage, the continuation of water stress and its negative impact caused an increase in carotenoids content again so the higher than recommended rates of N application (N4 and N5) positively affected the carotenoids content. An increase in carotenoids content under water stress conditions lead to an increased tolerance to water deficit [16]. In addition to their role in radiation absorption, carotenoids also protect the photosynthetic apparatus against the harmful free radicals [41].(Sifermann-Harms, 1987).

Soluble Sugars

Mean comparisons results showed that the soluble sugars content under all moisture conditions at 4-6 leaf stage was the highest with the application of 180 kg.ha-1 of nitrogen (control) and the production of soluble sugars was decreased by using nitrogen less or more than the recommended rate (figure 6a). The highest soluble sugars content (0.719 mg.g-1 FW) at 6-8 leaf stage was observed under favorable moisture conditions (70% of FC) and the application of N3 (control) (figure 6b). The lowest soluble sugars content (0.338 mg.g-1 FW) at the same stage was also observed under mild drought stress conditions (50% of FC) and the application of 270 kg.ha-1 of nitrogen (Figure 6b). Soluble sugars production was decreased under severe drought stress conditions (30% of FC) at all levels of nitrogen application during 8-10 leaf stage (figure 6c) compared to 6-8 leaf stage (figure 6b). Soluble sugars content production was also decreased under mild drought stress conditions (50% of FC) at all levels of nitrogen application except the N5 during 6-8 leaf stage (figure 6c) compared to 4-6 leaf stage (figure 6b).Studies done in recent years have emphasized on the role of sugars and sugar alcohols during drought stress conditions and changes in drought tolerance by manipulating the genes involved in the metabolism

of such compounds [8, 11, 17, 25, 47]. The main role of these sugars and sugar alcohols is the osmotic protection, osmotic adjustment, carbon storage and removing the radicals. The accumulation of soluble carbohydrates in response to environmental stresses is related to the osmotic adjustment or the protection of cellular membranes. Carbohydrates are able to play the role of metabolic signals, thus affecting the physiological response and the metabolic adjustment to the stress conditions [45]. Drought stress may lead to an increase in the hydrolysis of starch or a decrease in the transportation of sucrose, and leaf tissues may also act as osmotic protectors in cellular osmotic adjustment by maintaining the concentration of reducing sugars through their storage or synthesis [14]. The concentration of soluble sugars and proteins may also be varied among plants that are exposed to different moisture and nutritional conditions [32].Saneoka et al. (2004) stated that plants exposed to drought stress showed a higher adaptation to the stress at higher levels of nitrogen, and both the cell membrane stability and turgor pressure increased by an increase in nitrogen application. MDA concentration in leaves was also increased compared to control as a sign of lipid peroxidation under drought stress conditions but an increased application of nitrogen in plants under drought stress led to a decrease in MDA content [38]. They attributed drought tolerance to an increased use of nitrogen that prevented the damage to the cell membrane and increased the osmotic adjustment [38]. A decrease in soluble sugars content at higher levels of nitrogen application during 8-10 leaf stage in the current study, was consistent with the results reported by Zhou et al. (2011) stating a decrease in soluble sugars content due to an increase in the application of nitrogen [50]. A decrease in soluble sugars content through an increased application of nitrogen could therefore indicate that the nitrogen input allocated the organic carbon to a higher photosynthetic output for growth instead of developing the drought tolerance.

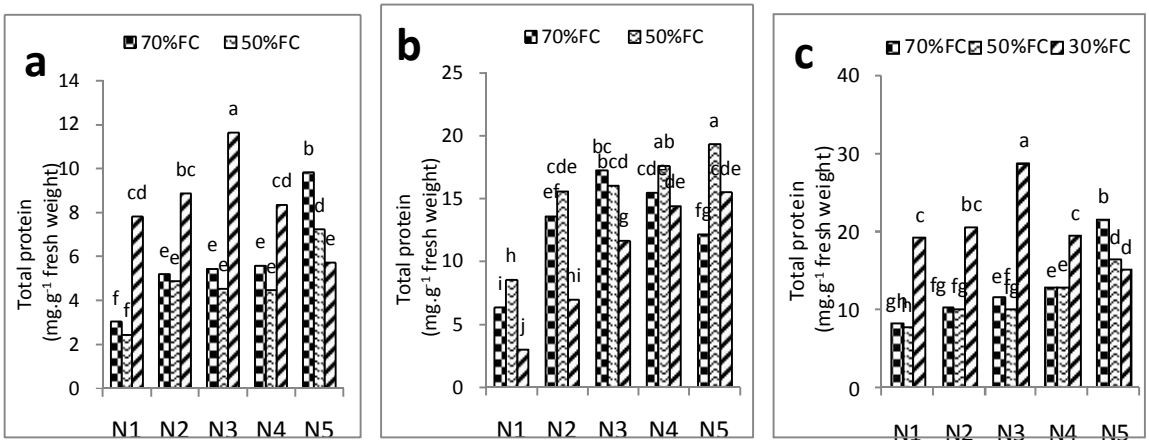

Figure 1-The interaction effect of drought stress and nitrogen on total protein at 4-6 (a), 6-8 (b) and 8-10 (c)leaf stages.

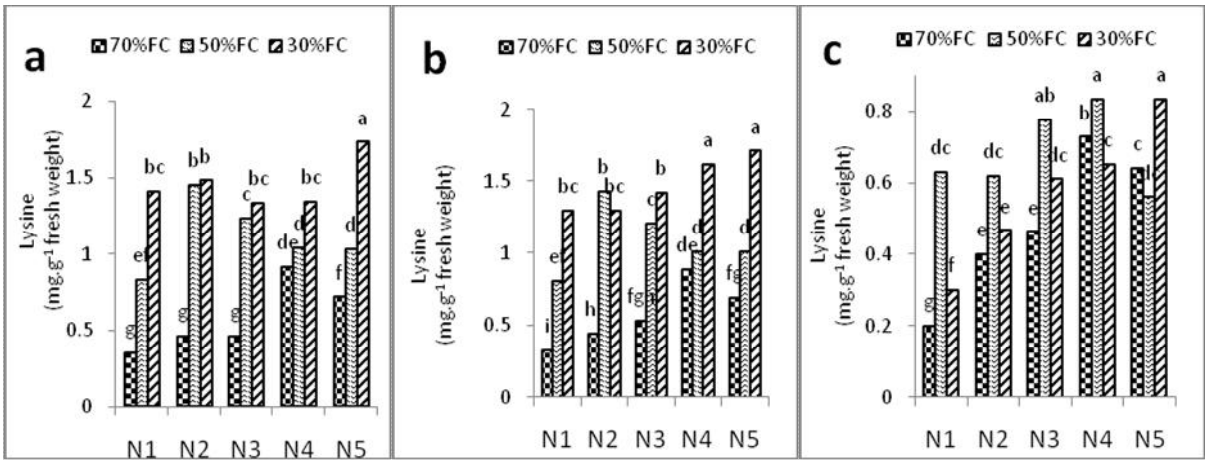

Figure 2-The interaction effect of drought stress and nitrogen on lysine at 4-6 (a), 6-8 (b) and 8-10 (c) leaf stages.

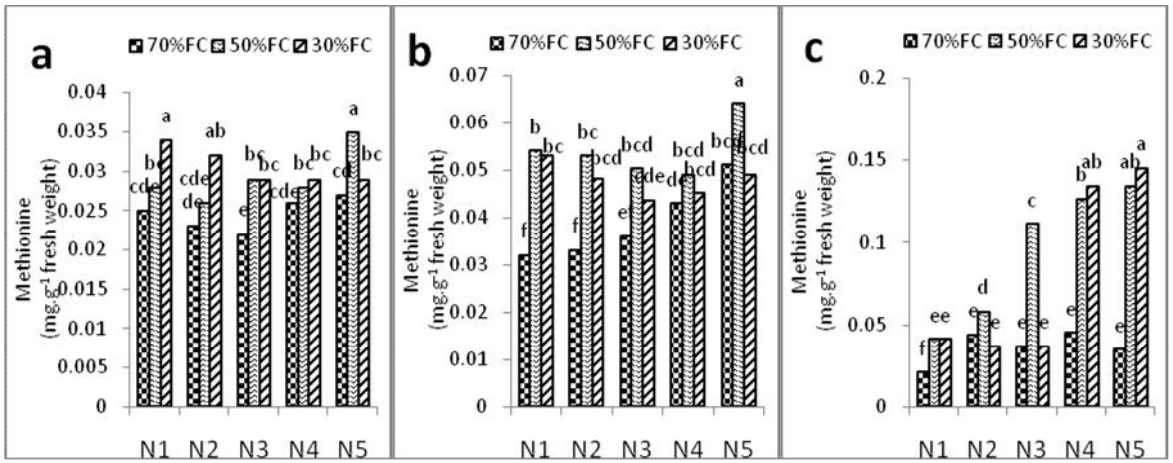

Figure 3-The interaction effect of drought stress and nitrogen on methionine at 4-6 (a), 6-8 (b) and 8-10 (c) leaf stages.

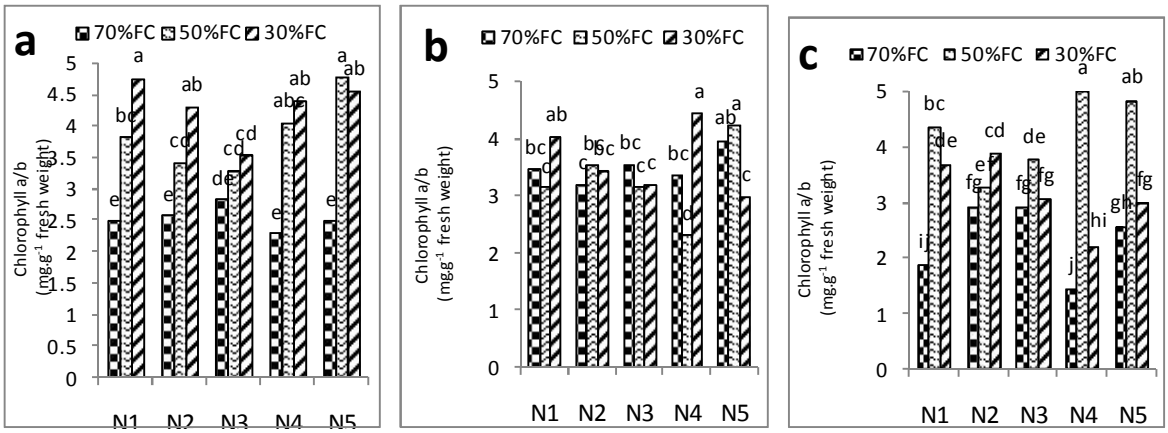

Figure 4- The interaction effect of drought stress and nitrogen on chlorophyll a/b at 4-6 (a), 6-8 (b) and 8-10 (c) leaf stages.

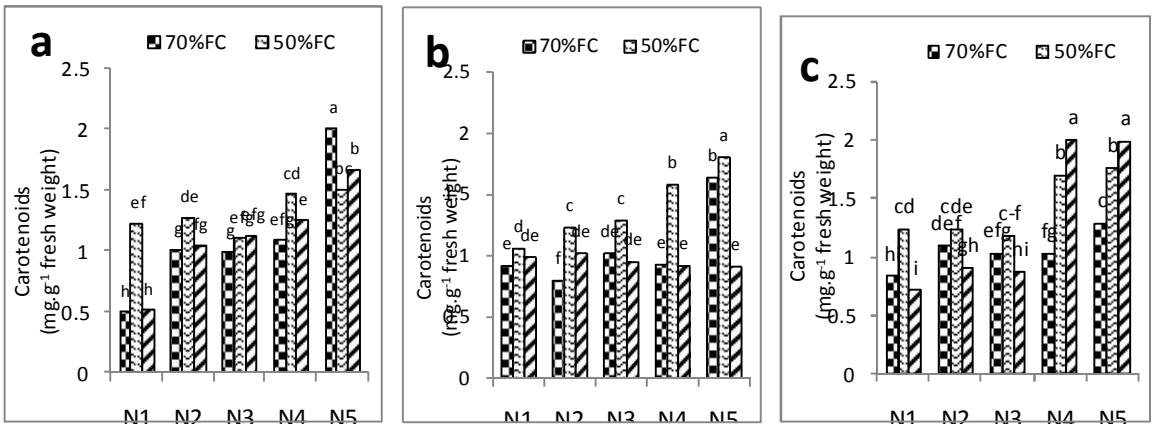

Figure 5- The interaction effect of drought stress and nitrogen on carotenoides at 4-6 (a), 6-8 (b) and 8-10 (c) leaf stages.

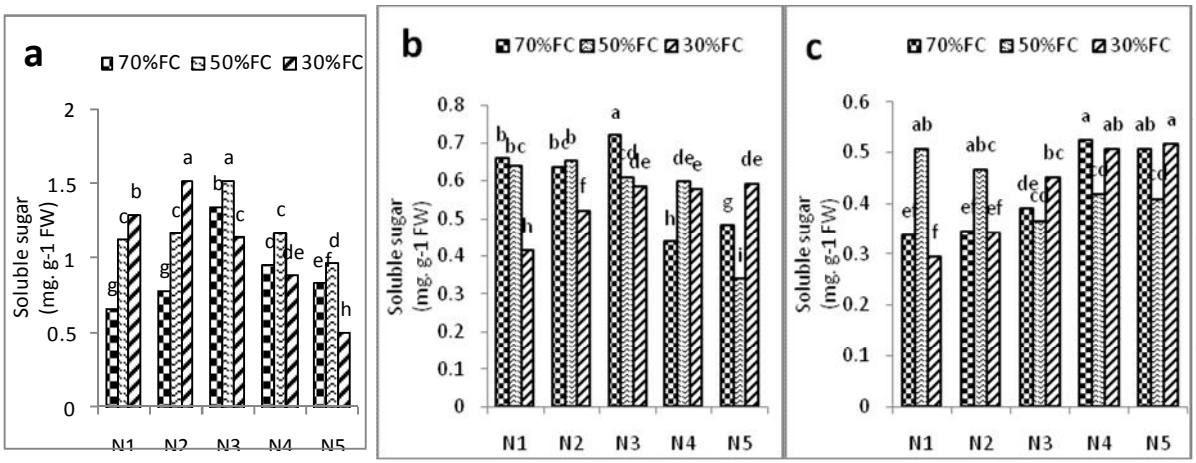

Figure 6- The interaction effect of drought stress and nitrogen on soluble sugars at 4-6 (a), 6-8 (b) and 8-10 (c) leaf stages.

CONCLUSION

Overall, it could be concluded that water stress and different levels of N application caused biochemical changes in canola leading to various responses from the plant's anti-oxidant system. In other words, canola seedlings used various mechanisms in their response to water stress and different N application rates.

REFERENCES

1. Anderson, G.Q.A., Andrews, M., Percival, S.M.,& Kirby, J.S.(1997). Nitrogen nutrition of brant (Brantabernicla L.) grazing on saltmarsh and pasture species. In: Proceedings of the XVIII International Grassland Congress, vol. 26 , pp. 3–4.
2. Andrews, M., Sprent, J.I., Raven, J.A.,&Eady, P.E.(1999). Relationships between shoot to root ratio, growth and leaf soluble protein concentration of Pisumsativum, Phaseolus vulgaris and Triticumaestivum under different nutrient deficiencies. Plant Cell Environ. 22: 949–958.
3. Anjum, S.A., Xie, X,& Wang, L. (2011). Morphological, physiological and biochemical responses of plants to drought stress. Afr. J. Agr. Res. 6: 2026-2032.
4. Antolin, M.C., Yoller, J.,& Sanchez-Diaz, M. (1995). Effects of temporary drought on nitrate-fed and nitrogen – fixing alfalfa plants. Plant Science. 107:159-165.
5. Arnon, A.N. (1967). Method of extraction of chlorophyll in the plants. Agronomy Journal. 23: 112-121.
6. Ashraf, M., & Harris, P.J.C. (2013). Photosynthesis under stressful environments: An overview. HOTOSYNTHETICA. 51 (2): 163-190.
7. Ashraf, M.,&Mehmood, S. (1990). Response of four *Brassica* species to drought stress. Environ. Exp. Bot. 30: 93-100.
8. Bartels, D., & R. Sunkar. (2005). Drought and salt tolerance in plants. Critical Reviews in Plant Sciences. 24(1): 23-58.
9. Baum, G., Lev-Yadun, S., Fridmann, Y., Arazi, T., Katsenelson, H., Zik, M.,& Fromm, H. (1996). Calmodulin binding to glutamate decarboxylase is required for regulation of glutamate and GABA metabolism and normal development in plants. EMBO Journal. 15: 2988-2996.
10. Chen, H. M., Muramoto, K., Yamauchi, F., &Nokihara, K. (1996).Antioxidant activity of designed peptides based on the antioxidativepeptide isolated from digests of a soybean protein. Journal of Agricultural and Food Chemistry, 44, 2619–2623.
11. Cook, D. S. Fowler, O. Fiehn, and M. F. Thomashow. 2004. A prominent role forthe CBF cold response pathway in configuring the lowtemperaturemetabolome of Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America, 101(42): 15243-15248.
12. Davies, M.J. (2005). The oxidative environment and protein damage. BiochimicaetBiophysicaActa. 1703: 93-109.
13. Estill, K., Delany, R.H., Smith, W.K.,&Ditterline, R.L. (1991). Water relations and productivity of alfalfa leaf chlorophyll variants. Crop Science. 31: 1229-1233.
14. De Souza, C. R.,Maroco, J. P., Dos Santos, T. P., Rodrigues, M. L., Lopes, C. M. , Pereira, J. S., & Chaves, M. M.(2005). Impact of deficit irrigation on water use efficiency and carbon isotope composition (delta C-13) of field-grown grapevines under Mediterranean climate. Journal of Experimental Botany. 56: 2163-2172.
15. Evans, J.R.(1989). Partitioning of nitrogen between and within leaves grown under different irradiances. Aust. J. Plant Physiol. 16: 533–548.
16. Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A. (2009). Plant drought stress: effects, mechanisms and management. Agronomy for Sustainable Development. 29: 185-212.
17. Garg, A. K, Kim, J. K., Owens, T. G.,Ranwala, A. P., Choi, Y. D. Choi,Kochian, L. V., & Wu, R. J. (2002). Trehalose accumulation in rice plants confers hightolerance levels to different abiotic stresses. Proceedings of the National Academy of Sciences. 99(25): 15898-15903.

18. Grumet R, Isleib T. G., Hanson, A. D. (1985). Genetic control of glycinebetaine level in barley. Crop Science. 25: 618-622.
19. Hajheidari, M., A. Eivazi, Buchanan, B.B., Wong, J. H., Majidi, I., &Salekdeh, G. H.(2007). Proteomics uncovers a role for redox in drought tolerance in wheat. Journal Proteome Research. 6: 1451-1460.
20. Hamilton, E. W.,&Heckathorn, S. A. (2001). Mitochondrial adaptations to NaCl, complex I is protected by anti-oxidants and small heat shock proteins, whereas complex II is protected by proline and betaine. Plant Physiology. 126: 1266-1274.
21. Hare, P.D.,& Cress, W.A. (1997). Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regulation. 21: 79-102.
22. Huseynova, M., Suleymanov, S.Y., Rustamova S.M., Aliyev. JA. (2009). Drought-induced changes in photosynthetic membranes of two wheat (*Triticumaestivum*L.) cultivars. – Russ. Biokhimiya 74: 1109-1116.
23. Je, J., Qian, Z., Byun, H., & Kim, S. (2007). Purification andcharacterization of an antioxidant peptide obtained from tunabackbone protein by enzymatic hydrolysis. Process Biochemistry. 42: 840–846.
24. Kannan, N.D.,&Kulandaivelu, G. (2011). Drought induced changes in physiological, biochemical and phytochemical properties of*Withaniasomnifera*Dun. – J. Med. Plants Res. 5: 3929-3935.
25. Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K., & Shinozaki, K. (1999). Improving plant drought, salt, and freezing tolerance by genetransfer of a single stress-inducible transcription factor. Nature biotechnology. 17(3): 287-291.
26. Kim, S. Y., Je, J. Y., & Kim, S. K. (2007). Purification andcharacterization of antioxidant peptide from hoki (Johniusbelengerii) frame protein by gastrointestinal digestion. Journal of Nutritional Biochemistry, 18, 31–38.
27. Klessig, D.F., Durner, J., Noad, R., Navarre, D.A., Wendehenne, D., Kumar, D., Zhou, J.M., Shah, J., Zhang, S.,&Kachroo, P. (2000). Nitric oxide and salicylic acid signaling in plant defense. Proceedings of the National Academy of Sciences of the United States of America. 97: 8849-8855.
28. Losak, T., Hlusek, J., Filipcik, R., Pospisilova, L., Manasek, J., &Prokes, K.(2010). Effect of nitrogen fertilization on metabolism of essential and non-essential amino acids yield-grown grain maize (*Zea mays* L). Plant Soil Environmental. 56: 574-579.
29. Martin-Tanguy, J. (2001). Metabolism and function of polyamines in plants: recent development (new approaches). Plant Growth Regulation. 34: 135-148.
30. McDonald, G.k.(1992). Effect of nitrogen fertilizer on the growth, grain yield and grain protein concentration of wheat. Crop Sci. 17: 791-793.
31. Munne-Bosch, S., Jubany-Mari, T.,&Alegre, L. (2001). Drought-induced senescence is characterizedby a loss of antioxidant defences in chloroplasts. Plant Cell Environ. 24: (13)1319–1327.
32. Nakaji, T.,Fukami, M.,Dokiya, Y., &Izuta, T. (2001). Effects of high nitrogen load ongrowth, photosynthesis and nutrient status of Cryptomeria japonica and Pinusdensiflora seedlings. Trees. 15(8): 453-461.
33. Niu, Y., Wang, Y., Li, P., Zhang, F., Liu, H., Zhang, G. (2013). Drought stress induces oxidative stress and the antioxidant defense system in ascorbate-deficient vtc1 mutants of Arabidopsis thaliana. ActaPhysiol Plant. 35:1189-1200.
34. Omokolo, N.D.,Tsala, N. G., andDjocgoue, P. F. (1996). Changes incarbohydrate, amino acid and phenol content in cocoa pods from three clonesafter infection with Phytophthoramegakarya Bra. andGrif. Annals of Botany: 77(2): 153-158.
35. Qasim, M., Ashraf, M., Ashraf, M.Y., Rehman, S.U.,&Rha, E. S. (2003). Saltinduced changes in two canola cultivars differing in salt tolerance. BiologiaPlantarum. 46: 629-632.
36. Rajapakse, N., Mendis, E., Jung, W. K., Je, J. Y., & Kim, S. K. (2005).Purification of a radical scavenging peptide from fermentedmussel sauce and its antioxidant properties. Food Research International. 38: 175–182.
37. Saiga, A., Tanabe, S., & Nishimura, T. (2003). Antioxidant activity ofpeptides obtained from porcine myofibrillar proteins by proteasetreatment. Journal of Agricultural and Food Chemistry. 51:3661–3667.
38. Saneoka, H.,Moghaieb, R.,Premachandra, G., & Fujita, K.(2004). Nitrogen nutrition and water stress effects on cell membrane stability and leaf water relations in *Agrostispalustris*Huds. Environmental and Experimental Botany. 52: 131–138.
39. Sarvajeet, S. G., &Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry. 48: 909-930.
40. Schwanz, P.,&Polle, A. (2001). Differential stress responses of antioxidative systems to droughtin pedunculate oak (Quercusrobur) and maritime pine (Pinuspineaster) grownunder high CO2 concentrations. J Exp Bot. 52:133–43.
41. Siefermann-Harms, D. (1987). The light-harvesting and protective functions of carotenoids in photosynthetic membranes. Plant Physiology. 69:561–568.
42. Sinclair, T. R., &Vadez, V.(2002): Physiological traits for crop yield improvement in low N and P environments. Plant Soil. 245: 1-15.
43. Sudhakar, C., Lakshmi, A., Giridarakumar, S.(2001). Changes in the antioxidant enzyme efficacyin two high yielding genotypes of mulberry (moursalba L.) under NaCl salinity, Plant Science. 161 (3): 613-619.
44. Sun, J.,Gu, J.,Zeng, J., Han, S., Song, A., Chen, F., Fang, W., Jiang, J., & Chen, S.(2013). Changes in leaf morphology, antioxidant activity and photosynthesiscapacity in two different drought-tolerant cultivars ofchrysanthemum during and after water stress. Sientia Horticulture. 161: 249-258.
45. Turkan, I. (2011). Plant Responses to drought and Salinity stress: Developments in a Post-Genomic Era (Vol. 57). Academic Press.

46. Umezawa, T., Fujita, M., Fujita, Y., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2006). Engineering drought tolerance in plants:discovering and tailoring genes to unlock the future. Current Opinion in Biotechnology. 17(2): 113-122.
47. Wang, J. S., Zhao, M. M., Zhao, Q. Z., & Jiang, Y. M. (2007).Antioxidant properties of papain hydrolysates of wheat gluten indifferent oxidation systems. Food Chemistry. 101: 1658–1663.
48. Xu, Z.Z.,& Zhou, G.S.(2006). Combined effects of water stress and high temperature on photosynthesis, nitrogen metabolism and lipid peroxidation of a perennial grass Leymuschinensis. Planta. 224 (5): 1080–1090.
49. Zhang, L. X., Li, S. X., Zhang, H., & Liang, Z. S. (2007). Nitrogen Rates and Water Stress Effects on Production, Lipid Peroxidation and Antioxidative Enzyme Activities in Two Maize (Zea mays L.) Genotypes. J. Agronomy & Crop Science. 193: 387—397.
50. Zhou, X., Zhang, Y.,Ji, X.,Dowing, A., &Serpe, M. (2011). Combined effects of nitrogen deposition and water stress on growth and physiological responses of two annual desert plants in northwestern China. Environmental and Experimental Botany. 74: 1-8.

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