ELBA BIOFLUX
Extreme Life, Biospeology & Astrobiology
International Journal of the Bioflux Society

Investigation on proline and carbohydrates accumulation in *Zea mays* L., under water stress condition

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Abstract. Water stress is considered as global concern in agricultural development. In order to investigate the effect of drought stress and different irrigation regimens on proline and carbohydrate accumulation as two main herbal compatibility mechanisms recent experiment was performed on *Zea* mays L., (Sc.704) in Agricultural Research Center located in Arsenjan city, Iran during summer 2011. The experimental design was split plot based on randomized complete blocks (RCB) in triplicates. In addition, drought stress as the main factor was induced at three growth stages named as vegetative, reproductive and seed filling (S1, S2, S3), respectively. The secondary factor was irrigation levels including 40, 70 and 100 mm accumulative evaporation from Pan Class A (I1, I2, I3). In the following the accumulative contents of proline and carbohydrates were determined in plant leaves. At different growth developments a significant increase in the contents of carbohydrates and proline was observed. As a conclusion, accumulation of proline and carbohydrates is considered as osmotic adjustment in response to drought stress condition.
Key Words: drought stress, *Zea mays* (Sc.704), praline, carbohydrates, irrigation regimens.

Introduction. There are many adaptive mechanisms found in plants in response to environmental changes such as drought stress. These mechanisms include changes in biochemical, morphological and physiological processes which naturally occur in plants. Accumulation of compatible solutes is considered as an important metabolic response to environmental changes which leads to metabolic adjustment. In these adjustments several organic materials such as sugars, polyols, betains and proline accumulate in plants (Yancey et al 1982).

The compatible solutes are divided into two categories: the first one includes compounds contain nitrogen group such as amino acids like proline apart from quaternary ammonium compounds, the second group contains constituents with hydroxyl groups such as sucrose, polyhydric alcohols and oligosaccharides (McCue & Hanson 1990). Previous studies state that osmotic adjustment is significantly influenced by accumulation of proline and carbohydrate in plants roots and shoots (Samaras et al 1995). Proline accumulation is a major response of higher plants, algae, animals and bacteria to low water availability (Delaauney & Verma 1993). Proline identical properties such as zwitterions, high hydrophilicity conveys this organic material an important compatible solutes which takes part as an osmoticum in plants (Samaras et al 1995). In addition, proline high concentration accumulation occurs in cell cytoplasm without any damage to cell structure and metabolism (Samaras et al 1995). Proline has been assigned the role of cytosolute a storage compounds or a protective agent for cytoplasmic enzymes and cellular structure (Demir 2000). Hanson & Hitz (1982) suggested that proline accumulation is a consequence of stress induced damaged to cells. In plants, the role of proline may not be restricted to that of a compatible osmolyte. Previous studies also demonstrate that accumulation in leaves in response to drought is the result of dramatic increase in biosynthesis and slower oxidation in mitochondria (Buitink et al 1998). On the other hand proline could cause macromolecules stabilizations apart from providing nitrogen and carbon supplement at water stress condition (Smirnoff & Cumbes 1989). Some experiments state radical detoxification role concerning with proline (Hare & Cress 1997). Carbohydrate accumulation is considerably occur in drought stress condition, for instance in grasses and cereals from Gramineae family during reproductive stage carbohydrate accumulation majorly takes place (Meier & Reid 1982). Carbohydrate storage is also considered as a response to environmental changes (Prado et al 2000). Carbohydrates play magnificent role in procedures such as synthesis of compounds, energy production, membrane stabilization (Hoekstra et al 2001), apart from gene expression mediator and molecular signaling (Smeekens 2000). The aim of present study is to elucidate *Zea mays* L. plant adaptive response to drought stress by determination and evaluation the contents of proline and carbohydrates as two well-characterized markers for osmotic adjustment.

Material and Method. This experiment was performed at Pharmacognosy Laboratory, Faculty of Pharmacy, Jundishapur University of Medical Sciences, Ahvaz, Iran, during summer 2011. One genotype of maize (*Z. mays*) – var. Sc.704 seeds were bought from Agricultural Research Center, Karaj, Iran. The seeds were cultivated in a farmland with the following characterizations in Aresenjan, Iran: 1500 km^2 surface area; approximately 1600 meter high from sea region (29°54′N, 53°18′E, 29.9°N, 53.3°E). The minimum and maximum cultivation temperature were 21.1 and 38.1^{o}C, respectively. The annual raining was 255.4 mm by average and it had a relative humidity of 36%. The studies were carried out in a split–plot, using Randomized Complete Block design with three replications. Treatment consisted of water stress at different growth stages of corn and it was confirmed at three levels. The main factors were included S1, S2, S3, defining as drought stress at vegetative, reproductive, grain filling stages of corn, respectively. Secondary factors included three irrigation levels I1, I2, I3 which implies as irrigation after 40, 70, 100 mm of cumulative evaporation, from Pan Class A, respectively. It should be pointed out that 27 plots were established initially according to experimental design study. Thus each experimental plot area had a surface area of 24 m^2, with 4x6 m dimensions and total area equals to 800 m^2. Each plot consisted of five plant lines and six meter length. In addition, the distance between main plots was three meters, whereas the plant distance on each row was 20 cm and the rows were 75 cm far from each other. It should be pointed that plant density per hectare comes to 66000. Plough, two vertical disks, leveling, furrow, mound were used regarding plot making. The soil texture was loamy silt clay as well (Table 1).

Table 1

Summary of soil physicochemical properties in Arsenjan region

Property	*0-30 cm depth*	*0-60 cm depth*
Saturation percentage	42	40
Electric conductivity (DSm^{-1})	0.73	1.72
pH	8.08	7.56
TNV (%)	58.4	20
Organic carbon (%)	1.02	0.3
Total nitrogen (%)	0.07	0.03
Potassium (ppm)	582	425
Phosphorous (ppm)	10	10.1
Clay (%)	21	24.8
Silt (%)	34	48.6
Sand (%)	49	29.6
Soil texture	Loamy	Silt clay
Iron	3.1	8.4
Zinc	0.7	1
Copper	0.62	1.4
Manganese	15	16

Frequent soil analysis was performed for determination of fertilizer content. Planting was accomplished after several ploughs. The grains used in this study were Hybrid single cross 704. The first irrigation was carried out in 27 June and thinning conducted in 4-5 leaf stages. The weeding was conducted in 2 stages of 20 and 40 days after planting, respectively. On the other hand, nitrogen fertilizer applied in two stages of 4-6 leafage and flowering time. Drought stress at vegetative, reproductive, grain filling stages with irrigation cut off through 14 days was performed. Drought stress induction at vegetative stage (S1) was performed concurrently to 4-5 leaf stage and followed up to the expression of tassel. Drought stress was continued until to the end of growth stage. On the other hand, drought stress was induced at reproductive stage (S2) from pollination up to the onset of seed filling stage. In addition, water stress induction at grain filling stage (S3) was carried out at the end of pollination and onset of seed filling process. It should be mentioned that plant water requirements was estimated on the basis of cumulative evaporation from Pan Class A. The irrigation was performed by application of poly ethylene tubes and water input was also monitored by a counter. In present experiment, final harvesting time was approximately in 14 October.

The amount of free proline in leaves was determined according to Bates method (Bates et al 1973). Dried leaves (0.5 g) were mixed with 10 ml sulfosalicylic acid 3% v/v. After filtration, it was centrifuged at 3000 g for about 25 minutes. The supernatant was mixed with acetic acid and Ninhydrin reagent. In the next step, the mixture was maintained in boiling water for approximately one hour. Then cooled and the absorbance was determined at 520 nm by UV-visible Spectrophotometer (CECIL, England, CE 250).

The amounts of carbohydrates were determined according to anthrone-sulfuric acid assay. The samples were defatted initially with diethyl ether, then at 80^{o}C soluble sugars were removed with ethanol 80%. The anthrone reagent was prepared by using Trevelyan and Harrison method (Money 1989). In the following, 0.2 g of 9, 10-dihydro-9-oxoanthracene (anthrone) dissolved in 100 ml of sulfuric acid, prepared by addition of 500 ml of concentrated acid to 200 ml purified water. In the next step, the reagent was stand for about 30-40 min, with shaking adequately until it was perfectly clear. This reagent was freshly prepared everyday and must be used within 12 hours. In the following, leaves extracts were prepared as explained by Yemen & Willis (1954). Leaves were exposed to ethanol 70% and extracted after evaporation and drying they were maintained in warm water and finally cleared with aluminium hydroxide. The samples were heated for 10 min then cooled. UV-Spectrophotometer was used at 620 nm for carbohydrate contents determination.

Statistical analysis. Statistical calculations were performed using ANOVA appropriate for RCBD with SAS ver. 9.1. In addition, analysis of variance was performed using MSTATC and MiniTab softwares. Excel software was used for charts adjustments as well. It should be pointed out for means comparison we applied DunCan's multiple range test at 0.05 probability levels when the F values were significant.

Results and Discussion. Variance analysis demonstrates that drought stress induction could significantly affect proline accumulation in *Z. mays* plant (Table 2). Results presented in Table 2 indicated that irrigation effects on proline concentration was significant at 1% level. In addition, it was seen interaction effect of drought stress × irrigation on proline concentration was not significant (Table 2). Present results showed the highest content of proline accumulation was in vegetative stage (S1) with average of 0.8236 mg and the minimum content was at seed filling stage (S3) with average content of 0.4626 mg (Figure 1). These results are in accordance with the findings of Mohammadkhani & Heidari (2008), Tatar & Gevrek (2008) and Kameli & Losel (1996) who reported that proline content increased under drought stress conditions. It should be noted that integration of drought stress on treatments was not significant (Table 2). Heuer (1994) reported that proline accumulation occurs in all plants organs, but proline accumulation in leaves is considerably greater in contrast to other plant parts. Proline is majorly stored in cell cytoplasm and probably it has protective role toward intracellular macromolecules during drought stress (Heuer 1994). Accumulation of proline and other amino acids in *Brassica napus* L. during drought stress induce water absorption in plant roots and results in growth continuity, although results in a decrease in plant yield (Good & Zaplachiniski 1994). In addition, the results indicate that a negative and significant correlation exists between carbohydrate and proline accumulation concerning with seed yield in *Z. mays* plant. Previous experiments demonstrate that accumulation of proline increases to a great extent at drought stress condition in *Pinus taeda* L. (Newton et al 1987). Accumulation of proline is considered as a response to water deficit and osmotic pressure increase (Safarnejad 1996). Proline has important role in herbal enzyme protections (Peng & Verma 1996), protein solubility maintainance (Walton & Podivinsky 1998), membrane phospholipid stabilization (Walton & Podivinsky 1998), cytoplasmic acidity adjustment (Peng & Verma 1996) and is a major source of cellular carbon and nitrogen (Walton & Podivinsky 1998). Mattioni et al (1997) state that salt stress impact on proline accumulation was much greater in *Triticum durum* L., in comparison to other amino acids contents. In addition, experiments on *Medicago sativa* L. plant illustrate that decrease in water potential to 1-2 MPa content cause a sudden and intensive increase in proline concentration concerning with phloem sap, they conclude that proline content in leaves increased and transferred to myrestimic tissues for osmotic adjustment (Mattioni et al 1997). There are many parameters involved in proline accumulation in response to water stress for example, ABA adjustment in optical procedures during proline metabolism (Girousse et al 1996), on the other hand scientists believe that some high energy compounds stimulate proline metabolism (Serraj & Sinclair 2002). Apart from that, drought stress could cause enzymes expression involved in proline synthesis reactions, however it can prevent proline destruction simultaneously (Hare et al 1999).

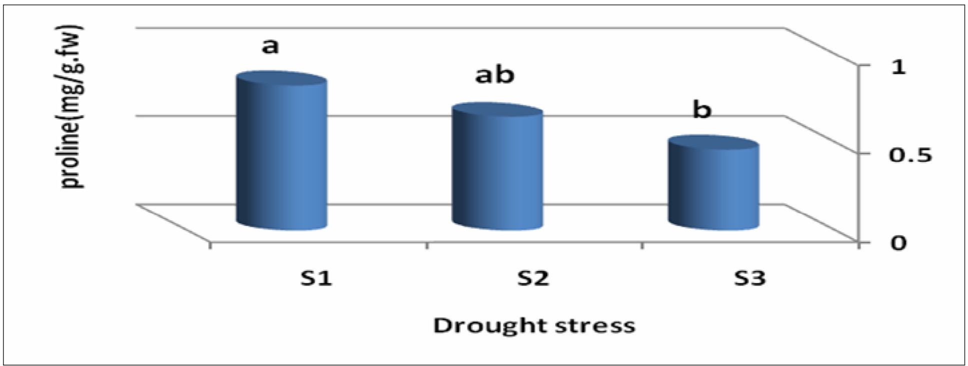

Figure 1. Effect of drought stress on different growth stages of corn leaves plant.

McMichael & Elmore (1977) reported that when predawn leaf water potentials reached -15 bars, leaf proline concentration increased sharply from trace to 86 m moles and to 160 m moles per g dry weight at -35 bars during the stress period. Proline increased slightly from 4.6-6.6 g/100g leaf protein.

Minimum content of proline accumulation was at 40 mm accumulated evaporation from Pan class A and maximum content was at 100 mm with the average of 0.5347 and 0.7881 mg, respectively (Figure 2). It should be mentioned that in some plants during water stress an increase in several amino acid contents occur but through long-term low water availability an increase in proline amount is observed in contrast to other types of amino acids (Rajinder 1987).

Different mechanisms are performed in plants for osmotic adjustment in response to drought stress. During osmotic adjustment plant has the opportunity for water absorption from root (Good & Zaplachiniski 1994). Carbohydrates have major role in osmotic adjustments since they are get involved in photosynthesis (Parakas et al 2002). Data presented in Table 2 illustrated that effect of drought stress on carbohydrate concentration was significant at 5% level. On the other hand, the results obtained from data variance analysis showed that irrigation effect on carbohydrate concentration was significant at 1% level. Results presented in Table 2 exhibited that drought stress × irrigation was not significant.

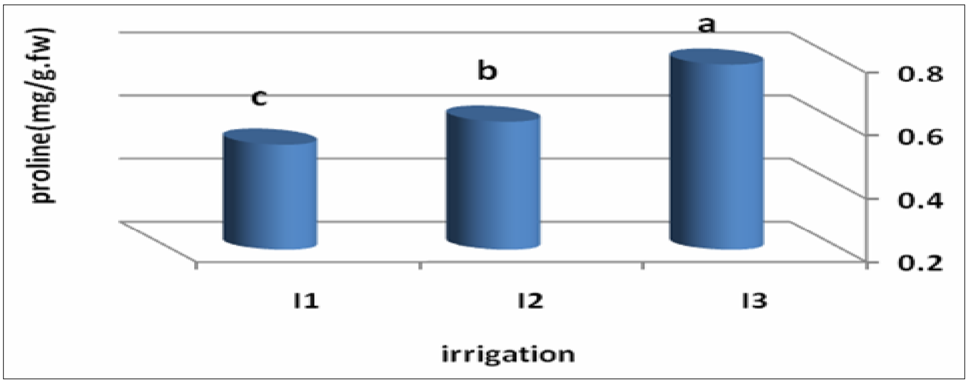

Figure 2. Effect of different irrigation levels on proline concentration in corn leaves plant.

Present results showed the maximum and minimum contents of carbohydrate accumulation was at vegetative (S1) and seed filling stages (S3) with the average contents of 67.57 and 66.05 mg, respectively (Figure 3). Therefore the results of present study are compatible to previous experiments and accumulation of carbohydrates could result in water potential decrease which is essential for growth development and physiological compatibility (Zinselmeier et al 1999; Wang et al 2000; Saeidi et al 2006).

In present study, carbohydrate accumulation was significantly affected by different irrigation regimens (Table 2). In addition, the minimum and maximum contents of carbohydrates belong to 40 and 100 mm accumulative evaporation from Pan class A, with average contents of 66.24 and 67.49 mg, respectively (Figure 4). Similar results were obtained by Khodary (2004) and El-Tayeb et al (2006). Therefore drought stress considerably affects carbohydrate metabolisms in plants. In addition, carbohydrates could take part in osmotic adjustment while they can cause membrane stability and herbal proteins protections (Ingram & Bartels 1996).

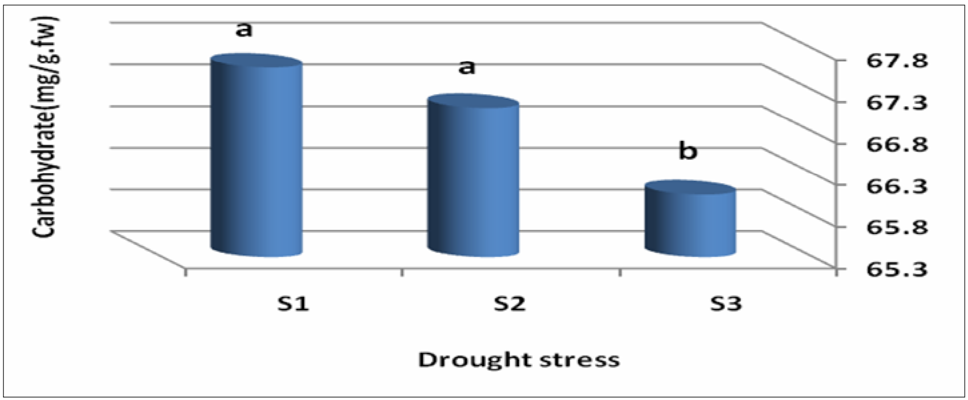

Figure 3. Effect of drought stress on different growth stages on carbohydrate in corn leaves plant.

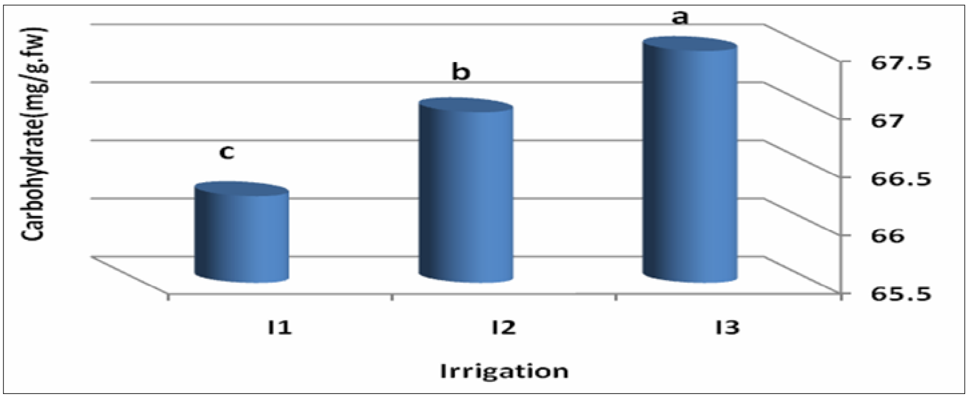

Figure 4. Effect of different irrigation levels on carbohydrate in corn leaves plant.

Table 2

Analysis of variance of investigated traits in corn (Sc.704) under both Irrigation levels and drought stress condition

		Mean squares (MS)	
SOV	*df*	*Proline (mg.g.fw)*	*Carbohydrate (mg.g.fw)*
Replication	2	0.1617**	0.5079^{ns}
Drought stress	2	0.8917*	16.3668*
Replication × drought stress	4	0.0887**	1.4884**
Irrigation	2	0.4600**	10.6595**
Drought stress × Irrigation	4	0.0398^{ns}	1.5407^{ns}
Replication × Irrigation	4	0.0250^{ns}	2.4170^{ns}
Error	62	0.0174	0.7665
CV (%)		20.5229	1.3086

ns, * and **: not significant, significant at the 5% and 1% levels of probability, respectively.

Conclusions. Drought is common phenomenon in present day agriculture. Much work has been done on alleviation of problem with plant improvement and using physical and chemical methods. But they are highly expensive, time consuming and do not meet the objective. Abiotic stress signaling is an important area with respect to increase in plant productivity. The knowledge generated through these studies should be utilized in making transgenic plants that would be able to tolerate stress condition without showing any growth and yield penalty. In recent study, the effects of drought stress and irrigation regimens on corn plant (SC.704) were significant. Concurrently management of maize plant in Arsenjan region with high potential of corn yield is inevitable.

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Received: 20 January 2013. Accepted: 28 February 2013. Published online: 30 March 2013.
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How to cite this article:
Azarpanah A., Alizadeh O., Dehghanzadeh H., 2013 Investigation on proline and carbohydrates accumulation in *Zea mays* L., under water stress condition. ELBA Bioflux 5(1):47-54.