Albanian j. agric. sci. 2013;12 (4): 653-657 *Agricultural University of Tirana*

RESEARCH ARTICLE **(Open Access)**

Various irrigation cycles effect on grain yield, proline and adaptive metabolits in some wheat genotypes

MAHDI ZARE[1*], MEYSAM SIASINEJAD[1], SASAN GHASEMI[2] AND ARASH AZARPANAH[2]

[1*]Department of Agriculture, Islamic Azad University, Abadeh branch, Abadeh, Fars, Iran

[2]College of Agriculture, Islamic Azad University, Shiraz Branch, Shiraz, Fars, Iran

Abstract:

Drought stress globally affects the growth and production of plants. Wheat is an important crop whose research in this aspect is highly noticeable related to food quality. In recent study, the effects of two different irrigation cycles (weekly (control) and 15-day irrigation) on leaf proline, carbohydrate, N, P, K accumulation and grain yield of three wheat genotypes (Chamran, Dehdasht and Kohdasht) were evaluated at the field of Agriculture Research Station of Borazjan, Bushehr state, Iran, during 2011-2012 growing season. Experimental design was split plot based on randomized complete block design in three replications. The results demonstrated that different irrigation cycles and wheat genotype effects were significant on leaf proline, carbohydrate, K leaf accumulation and grain yield. This finding suggested that Dehdasht genotype could be considered as more resistance genotype against drought condition than Kohdasht and Chamran genotypes. In arid condition which water is limited and dry land farming is necessary, Kohdasht could be selected as a tolerance genotype to water deficiency. Accumulation of proline and carbohydrates was also considered as osmotic adjustment in response to drought stress condition.

Key Words: Carbohydrate, drought stress, K accumulation.

1. Introduction

To improve crop productivity, it is necessary to understand the mechanism of plant responses to drought conditions with the ultimate goal of improving crop performance in the vast areas of the world where rainfall is limiting or unreliable. In addition, to the complexity of drought itself, plant's behavior responses to drought are complex and different mechanisms are adopted by plants when they encounter drought [7]. The mechanisms utilized by the plants for overcome the water stress effects might be via accumulation of compatible osmolytes, such as proline [3], carbohydrates accumulation [8], glycine betaine, and polyols in response to high salinity and drought stress conditions [22].

Proline plays a crucial role for cellular metabolism both as a component of proteins and as free amino acid. Due to its cyclic structure, proline has a restricted conformational flexibility, which determines the arrangement of the peptide chain around it, and as a consequence leads to stabilization or destabilization of secondary structures of protein conformation [16, 29]. 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 [3]. Carbohydrates play magnificent role in procedures such as synthesis of compounds, energy production, membrane stabilization [12], apart from gene expression mediator and molecular signaling [26].

The main aim of present study was to elucidate wheat (*Triticum aestivum* 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 to determine suitable selection criteria for selecting suitable genotypes under drought stress conditions.

2. Material and Methods

The present trial was conducted during 2011-2012 in the field of Agriculture Research Station of Borazjan, Bushehr state, Iran (29°16′N 51°13′E) with 80 meter elevated from sea level.

The assay was conducted under an average relative humidity of 70% and average minimum and maximum and temperatures and annual raining of 52.5 , -1°C and 550 mm, respectively. The soil was classified as a loamy silt clay soil comprised of 49% sand, 34% silt, and 21% clay with mean pH 8.8 and soil organic matter content of 1.2%. The experiment design was laid out entirely in a split–plot arranged in a randomized completed block design (RCBD) with three replications. Treatment consisted of water stress at different growth stages of wheat and it was

Correspondence: Mahdi Zare, Department of Agriculture, Islamic Azad University, Abadeh branch, Abadeh, Fars, Iran; Email: maza572002@yahoo.com
(Accepted for publication 9 November 2013)
***ISSN**: 2218-2020, © Agricultural University of Tirana*

confirmed at three levels. The main factors were included two irrigation cycles I_1: Control irrigation (weekly irrigation) and I_2: 15-day irrigation cycle, defining as drought stress. In recent study, sub plots were consisted of three wheat genotypes (V_1: Chamran, V_2: Dehdasht, V_3: Kouhdasht). Each experimental plot area had a surface area of $12m^2$, with 3×4 dimensions. Each plot was consisted of 12 plant lines and six meter length. In addition, the distance between main plots was estimated three meters, whereas the plant distance on each row was 20 cm and the rows were 25 cm far from each other.

Seeds were sown at 400 seeds m^2 on 12th October. Based on soil analysis, nitrogenous fertilizer as urea ($CO(NH2)_2$) was applied prior to planting, as topdressing at tillering stage and at flowering stage, 80 N kg ha^{-1} in each stage. During the growth period, all plots were weeded manually. The studied traits were leaf Phosphor (P), leaf potassium (K), leaf nitrogen (N), leaf proline and carbohydrate. The Kjeldahl method for determination of total N has been used. It was calculated by follows formula:

$$\%N = (T - B) \times N \times 1.401/ \text{g sample}$$

Where, T = mL of sample titrated, B = mL of blank titrated, N = acid normality

Samples should contain about 1 mg of N (no more than 5 mg) [6]. Determination of phosphorus in plant tissue by colorimetry was performed by Murphy and Riley [20] and Watanabe and Olsen [27] procedure. Determination of potassium in plants by atomic absorption techniques was carried out by Isaac and Kerber [13] procedure. Free proline accumulation was also determined using the method of Bates et al., [5]. Soluble sugars were determined based on the method of phenolsulfuric acid [10]. Contents of soluble sugar were expressed as mg g^{-1} FW. Starch content was also determined using the method of phenol-sulfuric acid [10]. Sediment of extract that filtered in sugar content dried, weighted and boiled with deionized water. Supernatant used for measurement of starch content. Skewness, kurtosis, homogeneity of variance and normality of data were tested by Minitab [17] statistical software. Analysis of variance of split-plot experiment based on RCBD and means comparison using Duncan's Multiple Range Test at P<0.05 were performed by SAS [23] and MSTATC [19] softwares.

3. Results and Discussion

The results indicated that the effect of irrigation on leaf K, proline, carbohydrate and grain yield was significant, indicating that these traits were influenced by drought stress condition. While, irrigation effect was not significant (*P*>0.05) for leaf P and leaf N contents, indicating that these traits were not influenced by drought stress condition (Table 1).

Table 1: Mean squares of studied traits in wheat

SOV	*df*	*Mean square (MS)*					
		Leaf P	Leaf K	Leaf N	Proline	Carbohydrate	Grain yield
B	2	0.00034 ns	0.00002 ns	0.15 ns	0.003 ns	2.72 ns	167.41 ns
I	1	0.00056 ns	0.00094 **	0.03 ns	44.9984 **	86528 **	103892.01 **
E (a)	2	0.00004	0.00001	0.09	0.0272	363.5	17.66
V	2	0.00169 **	0.02795 **	1.67 **	11.5046 **	886.22 **	54827.82 **
I×V	2	0.00016 **	0.00181 ns	0.86 **	3.7292 **	1814 **	23173.44 **
E (b)	8	0.00001	0.00064	0.05	0.017	5.86	297.54
CV%		2.36	7.22	9.2	3.16	0.32	2.84

ns, * and **: Not significant, significant at the 5% and 1% levels of probability, respectively.
SOV: Source of variation, df: degree of freedom, P: Phosphorus, K: Potassium, N: Nitrogen, B: Block, I: Irrigation, V: Variety

There were significant differences between genotypes for all the traits, indicating presence of genetic diversity among them for these traits (Table 1). Irrigation×variety interaction effects were also significant for all traits, except for leaf K content. Results of means comparison showed that the highest and lowest leaf phosphorus was related to Dehdasht and Kohdash, respectively (Table 2).

Interaction between 15-day irrigation cycle and Dehdasht genotype had the highest leaf phosphorus. In plants, the uptake of phosphorous was reduced under drought stress [4]. Phosphorous uptake decreased with decreasing soil moisture in wheat genotypes [2].

Results indicated that 15-day irrigation cycle had higher mean leaf K content compared with control irrigation. These findings were in accordance with those of Ashraf [2] who reported more potassium uptake under water deficit. During potassium deficiency, ion is transported from older leaves to the younger leaves and then to meristematic regions due to high mobility of K. Therefore, wheat plants may have accumulated potassium contents in developing ears (new growing sinks) for osmotic adjustment [28].

The results displayed that maximum and minimum leaf potassium content was belonged to Dehdasht and Kohdasht genotypes. As we know plants that have an optimum level of potassium in leaves will be more efficient in photosynthesis and become more resistant and tolerant to adverse conditions. The maximum and minimum leaf N contents were belonged to Chamran and Kohdasht genotypes, respectively. The results of interaction effects demonstrated that the plants under drought stress have high N level due to the accumulation of free amino acids that are not synthesized into protein because under drought stress nitrate reductase is affected adversely, which in the sequence of reactions is the first enzyme responsible for assimilation of nitrate into amino acid and in cell N compounds [25]. Thus the growth of cell and plant particularly leaves accompanied by nitrate accumulation in plant tissue is inhibited under water deficit [15].

Table 2. Effect of irrigation, variety and irrigation×varietyon studied traits in wheat

Treatment		*Leaf P (%)*	*Leaf K (%)*	*Leaf N (%)*	*Proline mg (g dw)$^{-1}$*	*Carbohydrate mg (g dw)$^{-1}$*	*Grain yield g m^{-2}*
Irrigation	I_1	0.15 a	0.34 b	2.59 a	2.55 b	823.44 a	684.22 a
	I_2	0.16 a	0.36 a	2.51 a	5.77 a	684.78 b	532.28 b
Variety	V_1	0.16 b	0.38 a	3.12 a	5.69 a	756.33 b	498.82 c
	V_2	0.17 a	0.40 a	2.45 b	3.65 b	765 a	675.47 a
	V_3	0.14 c	0.27 b	2.08 c	3.06 c	741 c	650.46 b
Interaction	$I_1 \times V_1$	0.15 c	0.36 a	3.57 a	3.21 d	806 c	507.26 c
	$I_1 \times V_2$	0.17 b	0.40 a	2.15 c	2.41 e	840.67 a	764.17 a
	$I_1 \times V_3$	0.14 d	0.28 a	2.05 c	2.03 f	823.67 b	781.23 a
	$I_2 \times V_1$	0.17 b	0.41 a	2.67 b	8.18 a	706.67 d	490.37 c
	$I_2 \times V_2$	0.18 a	0.40 a	2.75 b	4.88 b	689.33 e	586.76 b
	$I_2 \times V_3$	0.14 d	0.27 a	2.08 c	4.08 c	658.33 f	519.70 c

Means in each column, followed by similar letter(s) are not significantly different at 5% probability level, using Duncan's Multiple Range Test.
I_1: Control irrigation, I_2: 15-day irrigation cycle, V_1: Chamran,
V_2: Dehdasht, V_3: Kouhdasht, P: Phosphorus, K: Potassium, N: Nitrogen, dw: dry matter weight.

Results enumerated that maximum and minimum leaf proline contents were related to Chamran and Kohdasht genotypes. Colmer et al., [9] found that proline content was higher in sensitive wheat than in tolerant. However, the response varies depending on genotypes, intensity and duration of water stress [1]. In addition, it was seen increase of proline content in Chamran genotype was higher than in Kohdasht and Dehdasht genotypes under 15-day irrigation cycle. It is possible that these differences are due to up-regulation of proline degrading enzymes such as proline dehydrogenase (PDH) in drought stressed Chamran genotype. These results prove that proline accumulation by Chamran genotype is due to up-regulation of proline biosynthesis pathway rather than inhibition of catabolic process and that Chamran genotype to keep proline in a high level consume more energy and substances, but Dehdasht and Kohdasht genotypes keep their proline constant in a high level because of suitable management and resist in drought stress. Therefore, Chmran genotype had higher tolerance than Kohdasht and Dehdasht genotypes in severe drought stress. Our present results indicate that proline accumulation by repressed catabolic pathway under oxidative stress helps plants to decrease oxidative damage. These results were in accordance with the findings of Mohammadkhani & Heidari [18] who reported that proline content increased under drought stress conditions.

Present results exhibited that 15-day irrigation cycle had less content of carbohydrates compared with control irrigation. Therefore, drought stress considerably affects carbohydrate metabolisms in plants. The maximum and minimum carbohydrate contents were belonged to Dehdasht and Kohdasht genotypes, respectively. Accumulation of carbohydrates could result in water potential decrease which is essential for growth development and physiological compatibility [21].

Metabolisation of storage reserves in the endosperm of cereal seeds is tightly regulated and has a primary pivotal role in the interactions among sugars, ABA and gibberellins pathways responsible for the response to drought [11]. A central role of sugars depend not only on direct involvement in the synthesis of other compounds, production of energy but also on stabilization of membranes [12], action as regulators of gene expression [14] and signal molecules [26].

Dehdasht and Chamran genotype had the highest and lowest grain yield under control and 15-day irrigation cycles, respectively, indicating that

Dehdasht genotype was the best genotypes to increase grain yield.

4. Conclusions

This finding suggested that Dehdasht genotype could be considered as more tolerance genotype against drought stress condition than Kohdasht and Chamran genotypes. Based on this study, in arid condition which water is limited and dry land farming is necessary, Kohdasht could be selected as a tolerance genotype to water deficiency.

5. References

1. Abdalla MM, El-Khoshiban NH: **The influence of water stress on growth, relative water content, photosynthetic pigments, some metabolic and hormonal contents of *Triticum aestivum* cultivars.** *J. Appl. Sci. Res.,* 2007, 3: 2062-2074.

2. Ashraf MY: **Yield and yield components response of wheat (*Tritcium aestivum* L.) genotypes grown under different soil water deficit conditions.** *Acta Agron Hung.,* 1998, 46: 45-51.

3. Azarpanah A, Alizadeh O, Dehghanzadeh H: **Investigation on proline and carbohydrates accumulation in *Zea mays* L., under water stress condition.** *ELBA Bioflux* 2013, 5(1):47-54.

4. Baligar VC, Fageria NK, He ZL: **Nutrient use efficiency in plants.** *Communications in Soil Science and Plant Analysis* 2001, 32: 921-950.

5. Bates LS, Waldron RP, Teare ID: **Rapid determination of free proline for water stress studies.** *Plant Soil,* 1973, 39: 205-208.

6. Bremner JM, Mulvaney CS: **Nitrogen - Total.** In: *Methods of soil analysis:* AL Page et al. (ed.); 1982: 595-624.

7. Cattivelli L, Rizza F, Badeck W, Mazzucotelli E, Mastrangelo AM, Francia E, Mare C, Tondelli A, and Stanca M: **Drought tolerance improvement in crop plants: An integrated view from breeding to genomics**. Field Crops Res., 2008, 105(1-2): 1-14.

8. Chen M, Wei H, Cao J, Liu R, Wang Y, Zheng C: **Expression of Bacillus subtilis proBA genes and reduction of feedback inhibition of proline synthesis increases proline production and confers osmotolerance in transgenic Arabidopsis.** *J. Biochem. Mol. Biol.* 2007, 40: 396–403.

9. Colmer TD, Epstein E, Dvorak J: **Differential solute regulation in leaf blades of various ages in salt-sensitive wheat and a salt-tolerant wheat x *Lophopyrum elongatum* (Host) A. Love Amphiploid.** *Plant Physiol.,* 1995, 108:1715-1724.

10. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F: **Colorimetric method for determination of sugars and related substances.** *Anal. Chem.,* 1985, 38: 350-356.

11. Finkelstein RR, Gibson SI: **ABA and sugar interactions regulating development: Cross-talk or voices in a crowd.** *Curr. Opin. Plant Biol.,* 2001, 5: 26-32.

12. Hoekstra FA, Golovina EA, Buitink J: **Mechanisms of plant desiccation tolerance.** *Trends Plant Sci.,* 2001, 6: 431-438.

13. Isaac RA, Kerber JD: **Atomic absorption and flame photometry: Techniques and uses in soil, plant, and water analysis**.1971, p. 18-37. In: *Instrumental methods for analysis of soils and plant tissue:* LM Walsh. Soil Sci. Soc. Am., Madison, WI.

14. Koch K: **Carbohydrate-modulated gene expression in plants.** *Ann. Rev. Plant Physiol. Plant Mol. Biol.,* 1996, 47: 509-540.

15. Khondakar ZH, Aslam A, Rehman S, Khan TH: **Influence of soil moisture stress on yield, grain quality, availability and uptake of N, P and K by wheat.** *Int. J. Trop. Agr.,* 1983, 1: 211-220.

16. Miller G, Honig A, Stein H, Suzuki N, Mittler R, Zilberstein A: **Unraveling delta1-pyrroline-5-carboxylateproline cycle in plants by uncoupled expression of proline oxidation enzymes.** *J. Biol. Chem.* 2009, 284: 26482–26492.

17. Minitab: **MINITAB 12.** *Minitab, State College, PA*, 1998.

18. Mohammadkhani N, Heidari R: **Effects of drought stress on soluble proteins in two maize varieties.** *Turk. J. Biol.* 2008, 32:23-30.

19. MSTATC: **MSTATC a Microcomputer program for the design, management, and analysis of agronomic research experiments**. *Michigan State University, East Lansing, MI,* 1990.

20. Murphy J, Riley JP: **A modified single solution method for the determination of phosphate in natural waters.** *Anal. Chim. Acta.* 1962, 27:31-36.

21. Saeidi M, Moradi F, Ahmadi A, Poostini K, Najafian G: **Effect of exogenous application of ABA and CK at different stages of grain development on some physiological aspects of source and sink relationship in two bread**

wheat cultivars. *Iran. J. Crop Sci.,* 2006, 8:268-282.

22. Sarker AM, Rahman MS, Paul NK: **Effect of soil moisture on relative leaf water content, Chlorophyll, proline and sugar accumulation in wheat.** *J. Agron. Crop Sci.* 2009, 183: 225-229.

23. SAS I: **SAS user's guide. Version 8.** *SAS Institute Inc Cary, NC,* 2001.

24. Sinclair TR, Muchow RC: **System analysis of plant traits to increase grain yield on limited water supplies.** *Agron. J.* 2001, 93, 263–270.

25. Sinha SK, **Nicholas DJD: Nitrate reductase**. In: *The Physiology and Biochemistry of Drought Resistance in Plants.* LG Paleg, D Aspinall; 1981: 145-169.

26. Smeekens S: **Sugar-induced signal transduction in plants.** *Ann. Rev. Plant Biol.,* 2001, 51: 49-81.

27. Watanabe FS, Olsen SR: **Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soils.** *Soil Sci. Soc. Am. Proc.* 1965, 29:677-678.

28. Wignarajah K: **Mineral nutrition of plants**. In: *Handbook of Plant and Crop Physiology.* M Pessarakli; 1995: 193-222.

29. Xue X, Liu A, Hua X: **Proline accumulation and transcriptional regulation of proline biosynthesis and degradation in Brassica napus.** *BMB Rep.,* 2009, 42: 28–34.