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## Physiological response of amaranth (*Amaranthus caudatus* L.) to plant density under deficit irrigation

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### ABSTRACT

In order to evaluate the effects of water deficit and plant density on some physiological characteristics and biological yield of amaranth (*Amaranthus caudatus* L.), a factorial experiment was conducted based on Randomized Complete Block Design with three replications at the University of Tabriz in 2013. Treatments were irrigation intervals ( $I_1$  and  $I_2$ ; irrigations after 70 and 140 mm of evaporation from class A pan, respectively) and plant densities (40000, 80000 and 120000 plants/ha). The results showed significant interaction of irrigation  $\times$  plant density for leaf chlorophyll *a*, *b*, total chlorophyll, carotenoid, proline, relative water content (RWC), photosystem II efficiency (Fv/Fm) and biological yield. All chlorophyll content and biological yield increased with increasing plant density under water deficit. This increase was largely attributed to increased leaf thickness. Efficiency of photosystem II has positive correlation with relative water content and biological yield. The highest (41230.60 kg/ha) and lowest (14820.03 kg/ha) biological yield were respectively obtained from 120000 and 40000 plants/ha under deficit irrigation. © 2014 Trade Science Inc. - INDIA

### KEYWORDS

Chlorophyll content;  
Deficit irrigation;  
Photosystem II efficiency;  
Proline;  
Relative water content;  
Biological yield.

### INTRODUCTION

Among the environmental stresses, drought stress is one of the most adverse factors for plant growth and productivity<sup>[29, 28]</sup>. A small decrease in the availability of water to a growing plant immediately reduces its metabolic and physiological functions such as the decrease in chlorophyll contents, and an increase accumulation of proline in plants<sup>[32, 19]</sup>.

Grain and forage amaranth (*Amaranthus*

*caudatus*), as a plant possessing  $C_4$ -photosynthesis pathway, was also recognized as drought tolerant crop<sup>[8]</sup>. Anon<sup>[2]</sup>, reported that the  $C_4$  plant uses about three-fifth the amount of water a  $C_3$  plant uses to produce the same amount of plant materials because of the higher carbon dioxide uptake rates and greater stomatal resistance to water loss<sup>[16]</sup>. Drought tolerant crop can be characterized by growth response, changes in water relations of tissues exposed to low water potential, stomata conductance, ion accumulation, changes in fluorescence induction parameters

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and change of various gene expressions under water stress<sup>[11]</sup>. However, biosynthesis of proline, a well-known osmo-protectant, is triggered by drought stress and the expression level of the gene encoding pyrroline-5-carboxylate synthetase (P5CS), a component of proline synthetic pathway, is also increased<sup>[22, 39, 38]</sup>. Proline is accumulated in many plant species under various stress conditions<sup>[15]</sup>, as an osmo-regulator, maintain membrane integrity and affect the solubility of various proteins due to its interaction with hydrophobic residues on the protein surface under the conditions of reduced water availability<sup>[20]</sup>.

Variations in the chlorophyll contents are often measured, because its loss is often assumed to be a symptom of stress injury<sup>[25]</sup>. In *Brassica napus* cultivars, water stress reduced chlorophyll *a* + *b* content by 38% compared with the adequately watered plants<sup>[35, 26]</sup>. Another plant response to water deficit is change in photosystem II efficiency (Fv/Fm)<sup>[1]</sup>. The chlorophyll fluorescent measurements in field can reflect the exact response of photosynthetic apparatus which is more restricted under natural conditions<sup>[4]</sup>. Investigation based on assessments of chlorophyll *a* fluorescence have shown that PSII in quite resistant to water deficit, being either unaffected<sup>[34]</sup>.

Plant density is invariably linked with yield, the more plant stands up to a certain limit, the higher the expected yield<sup>[10]</sup>. The ideal plant number per unit area will depend on several factors, such as water availability and soil fertility<sup>[5]</sup>. Farmers thus face the problem of knowing the correct plant density to sow and also the exact amount (or optimum amount) of water to apply, which amount to apply in areas of abundance and areas of scarcity<sup>[33]</sup>.

Therefore, the present research was conducted to study the behavior of different plant densities of amaranth under water deficit stress conditions, with reference to the biochemical changes like chlorophyll and proline accumulation.

## MATERIALS AND METHODS

In order to study the effect of different plant densities of amaranth (*Amaranthus caudatus*) on the chlorophyll *a*, *b*, total chlorophyll, carotenoid, pro-

line, photosystem II efficiency (Fv/Fm), relative water content and biological yield under two irrigation (well watered and water deficit) condition, an experiment was conducted at the Research Farm of Tabriz university (Latitude 38°5' N, Longitude 46°17' E, Altitude 1360 m above sea level) in 2013. The experiment was arranged as factorial using Randomized Complete Block Design with three replications. Treatments were irrigation (I<sub>1</sub> and I<sub>2</sub>: irrigation after 70 and 140 mm of evaporation from class A pan, respectively) and plant density (40000, 80000 and 120000 plant per ha) were arranged as factorial experiment. All plots were irrigated immediately after sowing, and irrigation treatments were applied after seedling establishment. Weeds were controlled by hand weeding during crop growth and development. Some leaf physiological reaction to water deficit stress and plant density, leaf chlorophyll concentration<sup>[6]</sup>, carotenoid<sup>[24]</sup> and accumulation of leaf proline<sup>[27]</sup>.

Chlorophyll *a* (mg/g fresh weight) = { 12.7(663 nm) - 2.69(645 nm) } × V / (W × 1000)

Chlorophyll *b* (mg/g fresh weight) = { 22.9(645 nm) - 4.69(663 nm) } × V / (W × 1000)

Total chlorophyll (mg/g fresh weight) = { 20.2(645 nm) + 8.02(663 nm) } × V / (W × 1000)

Carotenoid (mg/g fresh weight) = { 1000(470 nm) - 1.82(Chl *a*) - 85.02(Chl *b*) / 198 } × V / (W × 1000)

Efficiency of photosystem II (Fv/Fm) was measured by a portable chlorophyll fluorometer (OPTISCIENCES, USA OS-30). Relative water content (RWC) was measured according to Ritchie *et al.*<sup>[30]</sup>, with the equation:

$$RWC = \frac{W_{\text{fresh}} - W_{\text{dry}}}{W_{\text{turgid}} - W_{\text{dry}}} \times 100$$

Variable included fresh weight of harvested leaves, which were cut to 1 cm segments (Wf); the weight of leaf segments soaked in water at 4°C in dark for 24h (Wt); and dry weight of the segments baked at 75°C for 24h (Wd).

At maturity, plants of 1 m<sup>2</sup> in the middle part of each plot were harvested and the biological yield was recorded. The data were analyzed by MSTATC software and the means were compared using Duncan

multiple range test at  $Pd''0.05$ .

**RESULTS**

Analysis of variance of the data showed the significant interaction effects of irrigation  $\times$  plant density on chlorophyll *a*, *b*, total, carotenoid, proline, relative water content, biological yield ( $Pd''0.01$ ) and Fv/Fm ( $Pd''0.05$ ) (TABLE 1).

Comparison of means indicated that chlorophyll *a*, *b*, total and carotenoid in limited irrigated plant were higher than those well irrigation at 80000 and 120000 density per ha (TABLE 2). The highest and the lowest chlorophyll *a* (0.008644, 0.005237 mg/g

fresh weight), *b* (0.003329, 0.002029 mg/g fresh weight), total (0.011978, 0.008218 mg/g fresh weight) and carotenoid (0.004854, 0.003056 mg/g fresh weight) belonged to plants irrigated after 140 mm of evaporation at 120000 plants/ha and 70 mm of evaporation at the same plant density, respectively (TABLE 2). Correlation coefficient between chlorophyll *a*, *b*, total and carotenoid were positive and significant (TABLE 3).

The results indicated that leaf proline under well watering increased with increasing plant density, in contrast under deficit irrigation decreased with increasing density (TABLE 2). Also, correlation between proline and carotenoid was negative and

**TABLE 1 : Analyses of variance of the effects of irrigation and plant density on some physiological characteristics and biological yield of Amaranth**

Source of variation	df	MS							
		Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid	Proline	Relative water content	Fv/Fm	Biological yield
Replication	2	0.0001 **	0.0001 **	39.164 **	0.0001 ns	0.0001 ns	19.936 ns	0.020 ns	1.580 ns
Irrigation (I)	1	782.761 **	70.646 **	884.297 **	37.469 **	0.001 **	120.798 **	0.130 **	22277.4 **
Density (D)	2	104.429 **	31.744 **	126.766 ns	30.347 **	0.015 **	702.830 **	0.183 **	7249122.7 **
I $\times$ D	2	533.945 **	109.249 **	706.619 **	318.260 **	0.127 **	2.733 **	0.055 *	1315620.9 **
Error	10	0.0002	0.0003	39.856	0.0002	0.0001	5.816	0.011	0.787
C.V (%)		0.02	0.06	6.03	0.03	0.25	8.56	15.55	0.03

ns, \* and \*\*: non-significant and significant at  $Pd''0.05$  and  $Pd''0.01$ , respectively

**TABLE 2 : Means of physiological traits for Amaranth affected by irrigation  $\times$  density interaction**

Different letters in each column indicate significant difference at  $Pd''0.05$

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**TABLE 3 : Correlation coefficients between physiological traits and biological yield of Amaranth plants under irrigation treatments and plant densities**

	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total chlorophyll	Carotenoid	Proline	RWC	Fv/Fm	Biological yield
Chlorophyll <i>a</i>	1							
Chlorophyll <i>b</i>	1.000**	1						
Total chlorophyll	0.943**	0.943**	1					
Carotenoid	0.943**	0.943**	0.829*	1				
Proline	-0.714	-0.714	-0.600	-0.886*	1			
RWC	0.371	0.371	0.600	0.086	0.143	1		
Fv/Fm	0.543	0.543	0.714	0.257	0.086	0.943**	1	
Biological yield	0.714	0.714	0.829*	0.543	-0.257	0.771	0.886*	1

\*, \*\*: Significant at  $Pd^*0.05$  and  $Pd^{**}0.01$ , respectively

statistically significant ( $r^2 = -0.886$ ) (TABLE 3).

Relative water content (RWC) increased with increasing density until 80000 plants/ha under both irrigation treatments (TABLE 2). In both irrigation treatments, maximum RWC was obtained at 80000 plants/ha that were statistically similar (TABLE 2).

Efficiency of photosystem II (Fv/Fm) plants, significantly increased with increasing water deficit, as maximum (0.755) and minimum (0.585) obtained under 140 mm and 70 mm evaporation from pan, respectively. The highest (0.8290) Fv/Fm obtained at 80000 plants/ha under 140 mm evaporation from pan that had not significant difference with  $I_1 \times D_2$ ,  $I_1 \times D_3$ ,  $I_2 \times D_1$  and  $I_2 \times D_3$  (TABLE 2). The correlation coefficient between Fv/Fm with RWC ( $r^2 = 0.943$ ) and Fv/Fm with biological yield ( $r^2 = 0.886$ ) were positive significant (TABLE 3).

Effect of irrigation  $\times$  density interaction on biological yield was also significant (TABLE 1). Biological yield increased up to about 80000 plants/ha and thereafter it was decreased (in well irrigated), but under deficit irrigation, biological yield increased with increasing plant densities (TABLE 2). The maximum (41230.60 kg/ha) and minimum (14820.03 kg/ha) yield, obtained at 120000 and 40000 plants/ha under deficit irrigation ( $I_2$ ) (TABLE 2). Correlation coefficient between biological yield and total chlorophyll were positive significant ( $r^2 = 0.829$ ) (TABLE 3).

## DISCUSSIONS

Decrease of chlorophyll concentration as an index for source evaluation<sup>[21]</sup>, can be consideration as a non-stomata limiting factor in the drought stress conditions. Resistant cultivar to drought and thermal stress conditions had high chlorophyll content compared with sensitive ones<sup>[31]</sup>. The higher photosynthetic pigments (chlorophyll *a*, *b*, total and carotenoid) obtained from 80000 and 120000 plants/ha under deficit irrigation (TABLE 2) may be resulted from reduction in specific leaf area under water deficit at all plant densities. Under water deficit condition, specific leaf area decreased with increasing plant density. Low water availability at high densities increases leaf thickness. Thicker leaves were the result of an increment in the size of palisade cells and also due to a major number of spongy parenchyma layers. The opposite behavior was the result of thinner leaves<sup>[3]</sup>. Therefore, at this situation concentration of chlorophyll per unit area increased.

In well irrigation regime, leaf proline increased along with increasing plant density (TABLE 2). So in this condition, due to restrictions on environmental resources such as water and nutrients, glutamate participates less to chlorophyll synthesis due to more proline synthesis or oxidative damage of chloroplast lipids, pigments and proteins<sup>[37]</sup>. But under water deficit proline decline with increasing plant density

(TABLE 2) that can be attributed to reduce cell size and keep turgor pressure and thus the stability of chlorophyll. But Azimi *et al.*<sup>[7]</sup> showed that proline content showed a pronounced increase by increasing the water stress levels of *Calendula officinalis* L. plants.

Relative water content is a reliable parameter for quantifying the plant water deficit stress response<sup>[9]</sup>. The increase in leaf RWC in water deficit condition at 80000 plants/ha (TABLE 2) could be related with high water availability in effect of a strong root systems and also small cell size. Small cells can withstand turgor pressure better than large cells, and can contribute to turgor maintenance more effectively under drought conditions<sup>[36, 13]</sup>.

Chlorophyll fluorescence analysis is a good index for measuring rapidly the change in photosynthetic metabolism of plants to such environmental stresses as drought<sup>[14, 18]</sup>. Fluorescence of chlorophyll reflected the photochemical activities of PSII<sup>[17]</sup>, with optimal values of around 0.832 measured from most plant species<sup>[23]</sup>. Values around this under stress condition indicated the plant has superior resistance to stress. The high efficiency of photosystem II under water deficit at 80000 density (0.8290) (TABLE 2) may be related to the high relative water content in this condition.

The high biological yield at 120000 plants/ha under water deficit condition (TABLE 2) can be attributed to high concentration of leaf chlorophyll (*a*, *b*, total) and carotenoid, increasing leaf area led to reduce evaporation from canopy in favor of transpiration. While high biological yield at 80000 plants/ha under well watering may be related to high relative water content. Similar results on forage maize under well watering reported by Budakli Carpici *et al.*<sup>[12]</sup>. They reported dry matter yield increased to maximum at 180000 plants/ha, but it declined as plant density increased further.

## CONCLUSION

These results indicate a close relationship between biological yield and plant density. Based on the results of this study it can be stated that the best density of amaranth under well watering and water

deficit condition were 80000 and 120000 plants/ha, respectively.

## REFERENCES

- [1] S.Ahmed, E.Nawata, M.Hosokawa, Y.Domae, T.Sakuratani; Alterations in photosynthesis and some antioxidant enzymatic activities of mung-bean subjected to waterlogging, *Plant Sci.*, **163**,117-123 (2002).
- [2] Anon; Publications on international agricultural research and development, International Rice Research Institute, Los Baños, Philippines, 539 (1984).
- [3] J.L.Araus, L.Alegre, L.Tapia, R.Calafell, M.D.Serret; Relationship between photosynthetic capacity and leaf structure in several shade plants, *American J.Bot.*, **73**, 1760-1770 (1986).
- [4] J.L.Araus, T.Amaro, J.Voltas, H.Nakkoul, M.M.Nachit; Chlorophyll fluorescence and selection criterion for grain yield in durum wheat under Mediterranean conditions, *Field Crops Res.*, **55**, 209-223 (1998).
- [5] G.Argenta, P.R.F.Silva, L.Sangoi; Arranjo de platas emmilho: Analise do estado da arte, *Ciencia Rural.*, **31**,1075-1084 (2001).
- [6] D.I.Arnone; Copper enzymes in isolated chloroplast polyphenol oxidase in *Beta vulgaris*, *J.Plant Physiol.*, **24**, 1-15 (1940).
- [7] J.Azimi, A.R.Pirzad, H.Hadi; Effect of increasing severity of drought stress on leaf physiological and morphological characters in *Calendula officinalis* L, *Bio Technol.Indian J.*, **6**, 212-218 (2012).
- [8] F.Bavec, M.Bavec; Grain amaranths, in *Organic production and use of alternative crops*, Boca Raton, CRC Press/Taylor and Francis Group., 88-107 (2006).
- [9] T.Y.Bayoumi, M.H.Eid, E.M.Metwali; Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes, *African J.Biotechnol.*, **7**, 2341-2352 (2008).
- [10] L.Bertoia, R.Burak, A.Nivio; Effect of plant densities on yield and quality of forage maize, *Maize Growers Co-operative Newsletter*, Santa Catarina, Brazil (1998).
- [11] A.Blum; *Plant breeding for stress environments*, Boca.Raton, Florida: CRC Press (1988).
- [12] E.Budakli Carpici, N.Celik, G.Bayram; Yield and quality of forage maize as influenced by plant Density and nitrogen rate, *Turkish J.Field Crops.*, **15**,

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- 128-132 (2010).
- [13] M.Burghardt, A.Burghardt, J.Gall, C.Rosenberger, M.Riederer; Physiological adaptations of water relations of *Teucrium chamaedrys* L. to the hot and dry climate of xeric limestone sites in Franconia (Southern Germany), *Flora.*, **203**, 3-13 (2008).
- [14] J.P.Conroy, R.M.Smillie, M.Kuppers, D.I.Bevege, E.W.Barlow; Chlorophyll fluorescence and photosynthetic and growth responses of *Pinus radiata* to phosphorus deficiency, drought stress and high CO<sub>2</sub>, *Plant Physiol.*, **81**, 423-429 (1986).
- [15] A.J.Delauney, D.P.S.Verma; Proline biosynthesis and osmo-regulation in plants, *Plant J.*, **4**, 215-223 (1993).
- [16] J.R.Ehleringer, T.E.Cerling, B.R.Helliker; C<sub>4</sub> photosynthesis, Atmospheric CO<sub>2</sub> and Climate. *Oecologia.*, **112**, 285-299 (1997).
- [17] R.A.Ganivea, S.R.Allahverdiyev, N.B.Guseinova, H.I.Kavakli, S.Nafisi; Effect of salt stress and synthetic hormone polystimuline K on the photosynthetic activity of cotton (*Gossypium hirsutum*), *Turkish J.Botany.*, **22**, 217-221 (1998).
- [18] B.Genty, J.M.Briantais, J.B.V.Da Silva; Effects of drought on primary photosynthetic processes of cotton leaves, *Plant Physiol.*, **83**, 360-364 (1987).
- [19] Y.Gibon, R.Sulpice, F.Larher; Proline accumulation in canola leaf discs subjected to osmotic stress is related to the loss of chlorophylls and to the decrease of mitochondrial activity, *Physiol.Planta.*, **4**, 469-476 (2000).
- [20] P.D.Hare; Molecular characterization of the gene encoding  $\Delta$  1-pyrroline-5-carboxylate reductase isolated from *Arabidopsis thaliana* (L.) Heynh.M.Sc.Thesis, Natal University, South Africa, (1995).
- [21] H.Herzog; Source and sink during the reproductive period of wheat, Scientific Publication, Berlin and Hamburg, (1986).
- [22] M.Ishitani, T.Nakamura, S.Y.Han, T.Takabe; Expression of the betaine aldehyde dehydrogenase gene in barley in response to osmotic stress and abscisic acid, *Plant Mol.Biol.*, **27**, 307-315 (1995).
- [23] G.N.Johnson, A.J.Young, J.D.Scholes, P.Horton; The dissipation of excess excitation energy in British plant species. *Plant, Cell and Environ.*, **16**, 673-679 (1993).
- [24] H.K.Lichtenthaler; Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, *Methods in Enzymol.*, **148**, 350-382 (1987).
- [25] S.S.Majumdar, B.Ghosh, R.Glick, E.B.Dumbroff; Activities of chlorophyllase, phosphoenol pyruvate carboxylase and ribulose-1, 5-bisphosphate carboxylase in the primary leaves of soybean during senescence and drought, *Physiol.Planta.*, **81**, 473-480 (1991).
- [26] R.Paclik, L.Sakova, V.Curn; Reaction of different cultivars of *Brassica napus* subs poleifera to water stress, *Fytotechnicka-Rada.*, **1**, 55-62 (1996).
- [27] R.Paquin, P.Lechasseur; Observation sur une methode de dosage de la proline libre dans les extraits de plantes, *Canadian J.Bot.*, **57**, 1851-1854 (1979).
- [28] A.R.Reddy, K.V.Chiatanya, M.Vivekanandan; Drought induced responses of photosynthesis and antioxidant metabolism in higher plants, *J.Plant Physiol.*, **161**, 1189-1202 (2004).
- [29] T.Y.Reddy, V.R.Reddy, V.Anbumozhi; Physiological responses to groundnut (*Arachis hypogea* L.) to drought stress and its amelioration, A critical review. *Plant Growth Regul.*, **41**, 75-88 (2003).
- [30] S.W.Ritchie, H.T.Nguyen; Leaf water content and gas exchange parameters of two wheat genotypes differing in drought resistance, *Crop Sci.*, **30**, 105-111 (1990).
- [31] R.K.Sairam, D.S.Shukla, D.C.Saxena; Stress induced injury and antioxidant enzymes in relation to drought tolerance in wheat genotypes, *Biol.Planta.*, **40**, 357-364 (1997/1998).
- [32] L.Sakova, R.Paclik, V.Curn; The drought tolerance of four Brassica species, *Sbornik -Jihoceska- Univerzita-Zemedelska-Fakulta,- Ceske-Budejovice.- Fytotechnicka-Rada.*, **1**, 77-86 (1995).
- [33] B.M.Sani, K.O.Oluwasemire, H.I.Mohammed; Effect of irrigation and plant density on the growth, yield and water use efficiency of early maize in the Nigerian savanna, *ARNP.N.Agric.Biol.Sci.*, **3**, 33-40 (2008).
- [34] Z.P.Shangguan, M.G.Shao, J.Dyckmans; Effects of nitrogen nutrition and water deficit on net photosynthesis rate and chlorophyll fluorescence in winter wheat, *J.Plant Physiol.*, **156**, 46-51 (2000).
- [35] K.D.Sharma, M.S.Kuhad, A.S.Nandwal; Influence of K nutrition on Brassica genotypes in response to water stress, *Plant Physiol.Biochem.*, **2**, 110-115 (1993).
- [36] E.Steudle, U.Zimmermann, U.Luttge; Effect of turgor pressure and cell size on the wall elasticity of plant cells, *Plant Physiol.*, **59**, 285-289 (1977).
- [37] E.A.Tambussi, C.G.Bartoli, J.Bettran, J.J.Guiamet, J.C.Araus; Oxidative damage to thylakoids proteins in water stressed leaves of wheat (*Triticum aestivum*

- L.), *Physiol.Planta.*, **108**, 398-404 (2000).
- [38] A.Ueda, W.M.Shi, K.Sanmiya, M.Shono, T.Takabe; Functional analysis of salt-inducible proline transporter of barley roots, *Plant Cell Physiol.*, **42**, 1282-1289 (2001).
- [39] Y.Yoshiba, T.Kiyosue, T.Katagiri, H.Ueda, T.Mizoguchi, S.K.Yamaguchi, K.Wada, Y.Harada, K.Shinozaki; Correlation between the induction of a gene for delta 1-pyrroline-5-carboxylate synthetase and the accumulation of proline in *Arabidopsis thaliana* under osmotic stress, *Plant J.*, **7**, 751-760 (1995).