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Response of Common bean (*Phaseolus vulgaris* L.) to Exogenous Application of Salicylic Acid (SA) under Water Stress Conditions

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ABSTRACT

Drought is an important abiotic factor that limits plant growth and productivity. Salicylic acid (SA) is a naturally existing phenolic compound. It is included in the group of plant growth regulators that regulates a large variety of physiological processes in plants. Recent studies have demonstrated the major role of SA in modulating plant responses to biotic and abiotic stresses such as drought. An experiment was, therefore, conducted to determine the effects of SA application on the growth and yield of common bean under different irrigation levels during 2011 in Iran. The experiment was laid out in a split plot on the basis of randomized complete block design with four replications. Two irrigation levels (I0= Irrigation after 50 mm evaporation from class A pan as no water stress and I1= Irrigation after 100 mm evaporation from class A pan as water stress conditions) were assigned to main plots and five levels of exogenous SA application (6 hours seed soaking in SA with concentrations of 0, 0.25, 0.5, 0.75 and 1 mM) were allocated to sub plots. Water stress reduced total chlorophyll content, stomatal conductance, net photosynthetic rate, relative water content, number of pods per plant, number of seeds per pod, 100-seeds weight and finally seed yield of common bean. Exogenous application of SA (especially 0.5 mM) improved all measured traits under both well watered and water stress conditions. Results signify the role of SA in regulating drought response of plants and suggest that SA could be used as a potential growth regulator, for improving common bean growth under water stress conditions.

Key words: *Common bean, Drought, Photosynthesis, Salicylic acid (SA), Yield*

Introduction

Water stress is one of the most common environmental stresses that affects growth and development of plants [1,2]. Drought is a permanent constraint to agricultural production in many developing countries and an occasional cause of losses of agricultural production in developed ones [3]. Drought stress is characterized by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata, and decrease in cell enlargement and growth [4,5]. Water stress inhibits cell enlargement more than cell division. It reduces plant growth by affecting various processes such as photosynthesis, respiration, translocation, ion uptake, nutrient metabolisms, biosyntheses of proteins, carbohydrates and growth promoters [2,4]. Common bean (*Phaseolus vulgaris* L.) is important protein source for many developing countries. As much as 60% of bean production the developing world occurs under conditions of drought stress [6]. This crop can be affected by several environmental stresses, and the drought is considered one of the most important causes of yield reductions. Common beans have low tolerance to water stress [7]. Water stress during the vegetative growth stage produced the shortest plants with the least leaf area. Soil water extraction and total water use were also least when water was withheld during the vegetative stage. Seed yield was reduced because of reductions in pods per plant or seeds per pod, when water stress occurred during the reproductive stage [8]. Singh [9] reported that water stress during flowering and grain filling reduced seed yield and seed weight and accelerated maturity of dry bean. Emam *et al.,* [10] found that plant height, number of leaves, leaf area, number of pods, pod dry weight and total dry weight of both common bean cultivars decreased significantly to water stress conditions. Find simple and practical methods to improve plant growth under drought conditions are very important. Salicylic acid (SA), an endogenous plant growth regulator has been found to generate a wide range of metabolic and physiological responses in plants thereby affecting their growth and development [11]. Salicylic acid has been found to play a key role in the regulation of plant growth,

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development, interaction with other organisms and in the responses to environmental stresses [12,13,14]. Further, its role is evident in seed germination, fruit yield, glycolysis, flowering in thermogenic plants [15], ion uptake and transport [16], photosynthetic rate, stomatal conductance and transpiration [17]. There are several reports available highlighting the role of SA to induce stress tolerance in plants [14,18]. SA has been found to induce heat tolerance in mustard [18,19], chilling tolerance in maize [20,21] and wheat [22], heavy metal stress tolerance in barley [23] and drought tolerance in wheat plants [24,25,26]. Senaratna *et al.*, [14] found that SA is one of the important signal molecules, which modulate plant responses to environmental stress. Hayat *et al.*, [27] studied the growth of water stressed tomato plants in response to exogenously applied salicylic acid. The results of their experiments revealed a significant decline in photosynthetic parameters, membrane stability index, leaf water potential, activities of the enzymes nitrate reductase and carbonic anhydrase, chlorophyll and relative water contents with a concomitant increase in proline content and the activities of antioxidant enzymes (CAT, POX and SOD). However, the treatment of these stressed plants with lower concentrations of salicylic acid significantly enhanced the aforesaid parameters thereby improved tolerance of the plants to drought stress. Khodary [28] reported increased chlorophyll and carotenoids contents in maize plants by SA application. SA application improved the performance of rice under both normal and water stress conditions [29]. Hussain *et al.*, [30] found that exogenous application of SA can improve yield and yield related traits in sunflower under drought stress. The lower concentrations of salicylic acid, when applied exogenously provided tolerance against the damaging effects of drought in tomato and bean plants, whereas, higher concentrations did not show fruitful results [14]. The objective of this study was to determine the effects of water deficit and exogenous application of salicylic acid on total chlorophyll content, stomatal conductance, net photosynthetic rate, relative water content, yield and yield components of common bean cv. Derakhshan in Iran.

Materials and Methods

This experiment was conducted at the research farm of the Islamic Azad University of Shahre-Rey in Tehran, Iran, during summer 2011. This region is located in an arid climate where the summer is hot and dry and the winter is cool and dry. Longitude, latitude and altitude of Shahre-Rey are 51 °28´ E, 35° 35´ N, and 1000m, respectively. The mean annual rainfall and temperature are 201.7 mm and 20.4 °c. Soil texture of research farm was sandy clay-loam with pH of 7.8, nitrogen 0.091%, phosphorus 9.1 ppm, potassium 350 ppm and EC of 2.8. The experiment was laid out in a split plot on the basis of randomized complete block design with four replications. Each replication had two main plots as irrigation levels viz, I0: Irrigation after 50 mm evaporation from class A pan and I1: Irrigation after 100 mm evaporation from class A pan, as control and water stress conditions, respectively. Each main plot consisted of five sub plots as common bean (cv. Derakhshan) seeds were soaked for 6 h in salicylic acid solutions (0, 0.25, 0.5, 0.75 and 1 mM). Seeds before treatment were sterilized with sodium hypochlorite solution (1%) for 5 min and then washed thoroughly with distilled water. Seeds were treated with Bavistin and then were sown by hand on 12 June 2011 in 4 cm depth of soil. At the same time plots were fertilized with 100 kg ha^{-1} ammonium phosphate. Each sub plot had four planting rows with length of 5 m thus, size of each plot was 10 m^2. Distances on and between rows were 10 and 50 cm respectively. All plots were irrigated immediately after sowing, but subsequent irrigations were carried out according to the treatments. Crop management practices such as hand weeding and thinning were done as required. At the flowering stage, total chlorophyll content, stomatal conductance, net photosynthetic rate and relative water content of each plot were measured as follows: Net photosynthetic rate (P_n) and stomatal conductance (g_s) were measured on sunny days between 11:00 and 12:00 hours on the youngest fully expanded leaves of 5 plants per plot by using a portable photosynthesis system (Handheld Photosynthesis System, CID Bio-Science CI-340, USA). Total chlorophyll content of the youngest fully expanded leaf of 5 plants per plot was measured by using the chlorophyll meter (Chlorophyll content meter, CL-01, Hansatech Instruments Ltd. England). The device readings were used as relative values for total chlorophyll content. To determine the relative leaf water content (RWC), 5 youngest fully expanded leaves of randomly selected 5 plants per plot were collected, immediately weighted to get fresh weight (Wf). Later these leaves were soaked in distilled water for 24 h and to record saturated weight (Ws), leaves were then dried in an oven at 80 ^oC for 48 h to record dry weight (Wd). RWC was calculated as: RWC = (Wf - Wd)/(Ws - Wd) × 100 %. At physiological maturity, 10 plants from each plot were harvested to determine pods per plant, seeds per pod and 100-seeds weight. Finally, plants in 2 m^2 of each plot were harvested and sundried, then, seeds detached from the pods and seed yield per unit area was recorded. Collected data were analyzed by MSTAT-C statistical software and the means were compared by Duncans Multiple Range test (DMRT) at the 5% probability level [31].

Results:

Total chlorophyll content:

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Drought significantly reduced relative total chlorophyll content. Nonetheless, seed soaking in SA (especially 0.5 mM) ameliorated this trait (Table 1). Exogenous SA improved chlorophyll content in non-stress and water stress conditions. The highest chlorophyll content (13.12) was achieved in control + SA application (0.5 mM) treatment, which was statistically at par with control + SA application at 0.75 mM, while, the lowest chlorophyll content (8.49) was observed in water stress + no application of SA treatment. In water stress conditions, the maximum chlorophyll content was produced in 0.5 mM SA application (Table 2).

Stomatal conductance (g_s):

Water stress significantly reduced stomatal conductance. This characteristic was improved by pre-treatment with SA (especially 0.5 Mm) (Table 1). In well watered and water stress conditions, application of SA, ameliorated g_s. The maximum g_s (628.0 mmol m^{-2} s^{-1}) was obtained in control + SA application (0.5 mM) treatment, while, the minimum g_s (387.0 mmol m^{-2} s^{-1}) was achieved in water stress + no application of SA treatment. In water stress conditions, the highest g_s was observed in 0.5 mM SA application, which was statistically similar to 0.75 mM (Table 2).

Net photosynthetic rate (P_n):

Water stress had negative effect on net photosynthetic rate. This attribute was markedly improved by the seed soaking in SA (especially 0.5 mM) (Table 1). In control and water stress conditions, application of SA, ameliorated P_n. The highest P_n (13.40 µmol co_2 m^{-2} s^{-1}) was recorded in control + SA application (0.5 mM) treatment, while, the lowest P_n (8.90 µmol co_2 m^{-2} s^{-1}) was produced in water stress + no application of SA treatment. In water stress conditions, the maximum P_n was obtained in 0.5 mM SA application (Table 2).

Relative water content (RWC):

Water deficit decreased considerably leaf relative water content. Nevertheless, application of SA (especially 0.5 mM) improved RWC (Table 1). Seed soaking in SA ameliorated RWC in well irrigated and water stress conditions. The maximum RWC (89.78%) was recorded in control + SA application (0.5 mM) treatment, against, the minimum RWC (60.15%) was produced in water stress + no application of SA treatment. In water stress conditions, the highest RWC was observed in 0.5 mM SA application, which was statistically at par with 0.75 mM (Table 2).

Number of pods per plant:

Drought stress had negative effect on number of pods per plant. This attribute was markedly improved by the seed soaking in SA (especially 0.5 mM) (Table 3). In control and water stress conditions, application of SA, ameliorated No. pods $plant^{-1}$. The highest No. pods $plant^{-1}$ (14.00) was recorded in control + SA application (0.5 mM) treatment, which was at par with control + SA application at 0.75 mM, against, the lowest No. pods $plant^{-1}$ (9.10) was produced in water stress + no application of SA treatment. In water stress conditions, the maximum No. pods $plant^{-1}$ was observed in 0.5 mM SA application, which was statistically at par with 0.75 and 0.25 mM (Table 4).

Number of seeds per pod:

Water shortage, decreased number of seeds per pod. However, pre-treatment with SA (especially 0.5 mM) ameliorated this trait (Table 3). In well irrigated and water stress conditions, application of SA, improved No. seeds pod^{-1}. The maximum No. seeds pod^{-1} (3.00) was obtained in control + SA application (0.5 mM) treatment, while, the minimum No. seeds pod^{-1} (2.30) was observed in water stress + no application of SA treatment. In water stress conditions, the highest No. seeds pod^{-1} was produced in 0.5 mM SA application, which was statistically similar to 0.75 and 0.25 mM (Table 4).

100-Seeds weight:

Water deficit reduced 100-seeds weight. Nonetheless, application of SA (especially 0.5 mM) improved this characteristic (Table 3). Exogenous SA ameliorated 100-seeds weight in well watered and water stress conditions. The highest 100-seeds weight (47.78 g) was achieved in control + SA application (0.5 mM) treatment, against, the lowest 100-seeds weight (42.88 g) was recorded in water stress + no application of SA treatment. In water stress conditions, the maximum 100-seeds weight was obtained in 0.5 mM SA application (Table 4).

Seed yield:

Water stress significantly decreased seed yield. Nevertheless, seed soaking in SA (especially 0.5 mM) ameliorated seed yield (Table 3). In control and water stress conditions, application of SA, improved seed yield. The maximum seed yield (401.3 g m^{-2}) was produced in control + SA application (0.5 mM) treatment, while, the minimum seed yield (179.4 g m^{-2}) was obtained in water stress + no application of SA treatment. In water stress conditions, the highest seed yield was recorded in 0.5 mM SA application, which was statistically similar to 0.75 and 0.25 mM (Table 4).

Discussion:

Drought is a major abiotic factor that limits agricultural crop production. It reduces plant growth by affecting various physiological and biochemical processes. The reactions of plants to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of growth [32]. Salicylic acid (SA) is an important signal molecule modulating plant responses to stress. It is recently reported to induce multiple stress tolerance in plants including drought [24].

The present study showed that water stress decreased total chlorophyll content. Nevertheless, seed soaking in SA improved this trait. Chlorophyll is one of the major chloroplast components for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation. Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers [33]. Loss of chlorophyll contents under water stress is considered a main cause of inactivation of photosynthesis. Furthermore, water deficit induced reduction in chlorophyll content has been ascribed to loss of chloroplast membranes, excessive swelling, distortion of the lamella vesiculation, and the appearance of lipid droplets [34]. These results are in line with those of Abass and Mohamed [35] and Abou El-Yazied [36] who observed that water stress decreased chlorophyll content of common bean. Similar to our results, Bideshki and Arvin [37] found that drought decreased total chlorophyll content in garlic but SA application improved this trait in both well watered and water stressed plants. Singh and Usha [24] also reported that the foliar application of SA in the range of 1–3 mM increased stomatal conductance, chlorophyll content and the activity of Rubisco compared with non-SA-treated wheat plants. Azooz and Youssef [38] reported that pretreatment of wheat with SA under drought stress, induced a significant stimulatory effect on the biosynthesis of photosynthetic pigments greater than estimated in untreated plants. This may be related to the inhibition of chlorophylase activity and chloroplast membrane degradation. Rhoads and McIntosh [39] observed marked increases in chlorophyll content and anthocyanins in SA treated *Spirodela* plants.

In our investigation, drought stress resulted in declined stomatal conductance (g_s) compared with well-watered conditions. While, decrease in the stomatal conductance was lower in SA-treated plants than control. Stomatal closure is one of the first responses to drought stress, and it is clear that stomata progressively close during the drought period [40]. Tesfaye *et al.*, [41], Ashraf and Iram [42] and Ramirez-Vallejo and Kelly [43] also found a significant decrease in stomatal conductance of common bean under water deficit conditions. In our study, exogenous SA application decreased reduction in stomatal conductance by drought. This correlates with the findings of Waseem *et al.*, [25] who observed that exogenous SA application causes an increase in stomatal conductance in wheat cultivars under drought stress compared to drought-stressed control. Saruhan *et al.*, [40] also reported that in the maize cultivars, stomatal conductance decreased significantly with increasing drought stress. Moreover, decreases in the stomatal conductance were lower in SA-treated plants than control for both cultivars. Our results suggest that SA pretreatment reverses drought-induced stomatal closure. This argument can be related to the findings of Rai *et al.*, [44] who observed that SA application can reverse the stomatal closure induced by ABA. However, Nemeth *et al.*, [45] reported that SA caused severe damage to photosynthesis in wheat plants subjected to drought stress by decreasing stomatal conductance and transpiration. They also reported higher electrolyte leakage in plants that were pre-treated with SA indicating serious membrane damage.

According to present experiment, under water deficit, net photosynthetic rate (P_n) was reduced significantly but application of SA ameliorated this characteristic. Under drought stress, photosynthesis is hampered mainly due to reduced stomatal conductance, changes in photosynthetic pigments and decreased activities of calvin cycle enzymes. Moreover, ROS production and imbalance between ROS and antioxidants. Under drought stress also disturb the photosynthetic apparatus and system [29]. Tesfaye *et al.*, [41] and Santos *et al.*, [46] also reported that net photosynthetic rate of common bean was reduced when water stress occurred. Nonetheless, many studies with different applications of SA have revealed a positive effect on photosynthesis and plant growth under drought stress. In our study SA application improved chlorophyll content, stomatal conductance and finally net photosynthetic rate of common bean in both non stress and water stress conditions. Similarly, Singh and Usha [24] reported that the foliar application of SA in the range of 1–3 mM increased stomatal conductance, chlorophyll content, activity of Rubisco and photosynthetic rate compared with non-SA-treated plants. Hamada and Al-Hakimi [47] showed that the treatment of wheat plants with 100 ppm SA through seed soaking was able to alleviate inhibitory effect of drought and stimulate growth by enhancing photosynthetic rate and reducing dark respiration. Khan *et al.*, [17] also reported that, foliar application of 10^{-5} mol/L SA caused 8 and 13% increases in photosynthetic rates of corn, 2 and 3 days after spraying, whereas 10^{-3} mol/L SA resulted an 8% increase 3 days after spraying. The photosynthetic rate of 10^{-5} mol/L treated soybean plants increased 6 to 11% from day 2 to day 5, after which it did not show any increase relative to the control plants. Zhou *et al.*, [48] showed that stem injection of corn with SA (10^{-2} mol/L from shortly after tasselling until physiological maturity) increased the photosynthetic rate.

Results of the present study showed that water stress reduced relative water content (RWC), while, seed soaking in SA improved RWC. Relative water content is considered as a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for identifying legumes with contrasting differences in dehydration tolerance [49]. RWC related to water uptake by the roots as well as water loss by transpiration. A decrease in the RWC in response to drought stress has been noted in wide variety of plants as reported by Nayyar and Gupta [50] that when leaves are subjected to drought, leaves exhibit large reductions in RWC and water potential. Exposure of plants to drought stress substantially decreased the leaf water potential, relative water content and Transpiration rate, with a concomitant increase in leaf temperature [51]. In agreement with the present experiment, De Mejia *et al.*, [52] and Ramirez-Vallejo and Kelly [43] also observed that drought, decreased RWC of common bean. In the present study, RWC declined in response to drought stress. On the other hand, in comparison with control plants, SA treated plants exhibited a slower decrease in RWC during drought stress. Similarly, Tari *et al.*, [53] found that exogenous SA treatment increased water potential and RWC of leaves of salt stressed tomato plants. Farooq *et al.*, [29] indicated that there was a strong correlation between plant water content and accumulation of compatible solutes (glycinebetaine and proline) under drought stress. Therefore, the improvement in RWC by exogenously application of SA may be a result of osmotic adjustment because of accumulation of compatible solutes like proline [54,55]. Indeed, our results (unpublished data) showed that proline content in SA treated plants was higher than control plants.

Results of our research showed that, water stress decreased significantly number of pods per plant, number of seeds per pod, 100-seeds weight and seed yield of common bean. Pre-treatment of seeds with SA showed significant increase in yield and yield components in both non stress and water stress conditions. Drought-related reduction in yield and yield components of plants could be ascribed to stomatal closure in response to low soil water content, which decreased the intake of CO_2 and, as a result, photosynthesis decreased [56,57,58]. In summary, prevailing drought reduces plant growth and development, leading to hampered flower production and grain filling and thus smaller and fewer grains. A reduction in grain filling occurs due to a reduction in the assimilate partitioning and activities of sucrose and starch synthesis enzymes [33]. Many studies similar to our experiment, reported that water stress reduces seed yield [43,52,59], pods number per plant[10,43,59], seeds number per pod and seed weight [43,59] of common bean. We observed that, exogenous SA application significantly improved yield and yield components of common bean in well watered and water stressed plants. Increase in seed yield seems to be the direct result of improved pods number per plant, seeds number per pod and 100-seeds weight. It may be the result of ameliorate of total chlorophyll content, stomatal conductance, net photosynthetic rate and relative water content owing to SA application. Similarly, Gomez *et al.*, [60] also observed an improvement in plant biomass and yield of wheat genotypes under water stress with SA application. Bideshki and Arvin [37] observed that drought, decreased yield of garlic but SA application improved this trait in both well watered and water stressed plants. Hussain *et al.*, [30] found that exogenous application of SA can improve yield and yield related traits in sunflower under drought stress. Khan *et al.*, [61] also revealed that application of 0.5 mM SA on mung bean grown under nonsaline conditions increased pod length by 19.9%, pod number by 19.9%, seed number by 20.2% and seed yield by 20.1% in comparison to the control.

Conclusion:

We found that water stress reduced total chlorophyll content, stomatal conductance, net photosynthetic rate, relative water content, yield and yield components of common bean. SA application improved all measured traits not only under well watered but also under water stressed plants. The effect of 0.5 mM SA was more considerable. The present investigation suggests that application of SA may help decrease the adverse effects of drought in common bean.

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Table 1: Effect of water stress and SA application on total chlorophyll content (TCC), stomatal conductance (g_s), net photosynthetic rate (P_n) and relative water content (RWC) of common bean.

Treatments	TCC (Device unit)	g_s (mmol m^{-2} s^{-1})	P_n (µmol co_2 m^{-2} s^{-1})	RWC (%)
Irrigation				
Control	12.41 a	577.4 a	12.28 a	87.46 a

Water stress	9.26 b	426.2 b	9.86 b	71.31 b
SA application (mM)				
0	9.83 e	444.5 e	10.05 e	72.34 e
0.25	10.75 c	499.0 c	10.85 c	80.00 c
0.5	11.63 a	545.5 a	12.00 a	82.67 a
0.75	11.49 b	535.0 b	11.80 b	82.25 b
1	10.48 d	485.0 d	10.65 d	79.67 d

Means with the same letter in each column and treatment are not significantly different at probability level of 5% using DMRT.

Table 2: Interaction effects of water stress and SA application on total chlorophyll content (TCC), stomatal conductance (g_s), net photosynthetic rate (P_n) and relative water content (RWC) of common bean.

Irrigation	SA (mM)	TCC (Device unit)	g_s (mmol m^{-2} s^{-1})	P_n (µmol co_2 m^{-2} s^{-1})	RWC (%)
Control	0	11.17 d	502.0 e	11.20 e	84.54 e
	0.25	12.56 b	576.0 c	11.90 c	87.23 c
	0.5	13.12 a	628.0 a	13.40 a	89.78 a
	0.75	13.00 a	611.0 b	13.20 b	89.01 b
	1	12.22 c	570.0 d	11.70 d	86.74 d
Water stress	0	8.49 i	387.0 i	8.90 j	60.15 h
	0.25	8.93 g	422.0 g	9.80 h	72.77 g
	0.5	10.14 e	463.0 f	10.60 f	75.56 f
	0.75	9.98 f	459.0 f	10.40 g	75.49 f
	1	8.73 h	400.0 h	9.60 i	72.59 g

Means with the same letter in each column are not significantly different at probability level of 5% using DMRT.

Table 3: Effect of water stress and SA application on yield and yield components of common bean.

Treatments	No. pods $plant^{-1}$	No. seeds pod^{-1}	100-Seeds weight (g)	Seed yield (g m^{-2})
Irrigation				
Control	12.84 a	2.82 a	46.57 a	339.0 a
Water stress	9.78 b	2.48 b	44.11 b	214.8 b
SA application (mM)				
0	10.35 c	2.49 e	44.03 e	230.1 e
0.25	11.50 b	2.67 c	45.43 c	281.9 c
0.5	12.15 a	2.78 a	46.28 a	318.7 a
0.75	11.90 a	2.73 b	46.19 b	305.3 b
1	10.65 c	2.58 d	44.78 d	248.6 d

Means with the same letter in each column and treatment are not significantly different at probability level of 5% using DMRT.

Table 4: Interaction effects of water stress and SA application on yield and yield components of common bean.

Irrigation	SA (mM)	No. pods $plant^{-1}$	No. seeds pod^{-1}	100-Seeds weight (g)	Seed yield (g m^{-2})
Control	0	11.60 c	2.68 e	45.18 e	280.9 e
	0.25	13.00 b	2.80 c	46.50 c	338.5 c
	0.5	14.00 a	3.00 a	47.78 a	401.3 a
	0.75	13.60 a	2.91 b	47.70 b	377.5 b
	1	12.00 c	2.71 d	45.69 d	297.1 d
Water stress	0	9.10 e	2.30 h	42.88 j	179.4 h
	0.25	10.00 d	2.54 f	44.36 h	225.3 f
	0.5	10.30 d	2.56 f	44.77 f	236.1 f
	0.75	10.20 d	2.55 f	44.68 g	233.2 f
	1	9.30 e	2.45 g	43.88 i	200.0 g

Means with the same letter in each column are not significantly different at probability level of 5% using DMRT.

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