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The Effect of Proline Application on Drought Tolerance of Cowpea (*Vigna unguiculata* L.)

Amir Aslan Ardabili, Omid Sadeghipour and Abolfazl Rashidi Asl

Department of Agronomy, Shahre-Rey Branch, Islamic Azad University, Tehran, Iran.

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ABSTRACT

Drought is an important abiotic factor that limits plant growth and productivity. Amino acid proline is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses. In order to investigate the effect of proline application on drought tolerance of cowpea an experiment was done in split plot at the basis of randomized complete block design with three replications at the Tehran, Iran during summer 2012. Irrigation as a major factor in two levels: irrigation after 50 mm evaporation from class A evaporation pan (normal conditions) and irrigation after 100 mm evaporation from class A evaporation pan (water stress) were the main plots. Proline application was used as a subplot in seven levels: no use of proline, seeds soaking for 12 h in proline concentration of 20 mM, seeds soaking for 12 h in proline concentration of 40 mM, seeds soaking for 12 h in proline concentration of 60 mM, spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 20 mM, spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 40 mM, spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 60 mM. The results showed that water stress, decreased yield and yield components, biomass, harvest index and plant height. Proline application had a positive impact on the measured traits under normal and water stress conditions. The highest seed yield (1008.96 g m^{-2}) was obtained in the combination treatment of irrigation after 50 mm evaporation and spraying of proline in two stages of 6-leafy and flowering in proline concentration of 20 mM and the lowest seed yield (489.88 g m^{-2}) was produced in the combination treatment of irrigation after 100 mm evaporation and no use of proline. The results revealed that the proline application at different levels, especially as foliar spraying at two stages of 6-leafy and flowering caused more production of cowpea under normal and water stress conditions.

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INTRODUCTION

Drought is a major limiting factor for crop production in the world [9]. Because water is essential at every stage of plant growth from seed germination to plant maturation [8,14]. 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 [28,36]. Keeping in view the considerable demand for food, crop improvement for drought stress tolerance is of prime importance [13,14]. In view of Serraj and Sinclair [46] osmotic adjustment is one of the major physiological phenomena vital for sustaining growth of plants under water stress. It has been widely reported that plants accumulate a variety of compatible solutes such as proline and betaine, as an adaptive mechanism of tolerance to salinity and drought [5, 6, 26, 43]. These compatible solutes protect and stabilize 3D structure of proteins and photosynthetic apparatus [41], regulate cellular osmotic adjustment [47,53] and detoxify reactive oxygen species (ROS) [5,12] in response to abiotic stresses. Upon relief from stress these solutes are metabolized and are considered as an important energy source for recovery from stress [25]. Although much attention has been paid on the role of proline in stress tolerance as a compatible osmolyte [17, 33, 35, 44, 54], little attention has been given on its role in other biochemical and physiological processes responsible for stress tolerance in plants [30,37, 39]. In response to drought or salinity stress in plants, proline accumulation normally occurs in the cytosol where it contributes substantially to the cytoplasmic osmotic adjustment [11, 29, 32]. Exogenous application of proline can play an important role in enhancing plant stress

Corresponding Author: Amir Aslan Ardabili, MSc. Department of Agronomy, Shahre-Rey Branch, Islamic Azad University, Tehran, Iran.
E-mail: amir_ardabilii@yahoo.com; Tel. +989125236783; Tel-Fax: +982155229283; P.O. Box: 18155/144, Tehran, Iran.

tolerance. This role can be in the form of either osmoprotection [24,52]. Cowpea (*Vigna unguiculata*) is an important tropical and subtropical grain legume providing protein, vitamins and minerals. It is a summer crop which produced by irrigation in Iran. There is competition for water by the agricultural, domestic and industrial users during the dry season, so there is the need to conserve and optimal use of the available water. Thus, finding simple, applicable and effective methods in improving crop tolerance to these stresses is important. Therefore, in this study we study the effect of exogenous application of proline in improving drought tolerance of cowpea.

MATERIALS AND METHODS

This experiment was conducted at the research farm of the Islamic Azad University of Shahre-Rey in Tehran, Iran, during summer 2012. 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 three replications. Irrigation as a major factor in two levels: irrigation after 50 mm evaporation from class A evaporation pan (control) (I_0) and irrigation after 100 mm evaporation from class A evaporation pan (water stress) (I_1) were the main plots. Proline application was used as a subplot in seven levels: no use of proline (p_0), seeds soaking for 12 h in proline concentration of 20 mM (P_1), seeds soaking for 12 h in proline concentration of 40 mM (P_2), seeds soaking for 12 h in proline concentration of 60 mM (P_3), spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 20 mM (P_4), spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 40 mM (P_5), spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 60 mM (P_6). Each sub plot had four planting rows with length of 4 m. Distances on and between rows were 10 and 50 cm respectively. Seeds of cow pea (cv. kamran) were sown by hand on 11 June 2012 in 4 cm depth of soil. First irrigation was after planting to provide favorable conditions for the growth of output and raising seedlings from the soil seed germination. During the 2 to 4 leaf stage, optimum plant density per unit area by removing excess plants and the distance between plants was 10 cm. Crop management practices such as hand weeding and thinning were done as required. First proline spraying on plant leaves in stages of 6-leafy was done in the morning before sunrise. And tried to spray under the leaves. As soon as the status of plants in most plots were in the middle of flowering, the second proline was spraying. At physiological maturity, 10 plants from each plot were harvested to determine pods per plant, seeds per pod, 1000-seeds weight and plant height. Plants in 1.5 m^2 of each plot were harvested and sundried, then, seeds detached from the pods and seed yield per unit area was recorded. Finally, that plants in 1.5 m2 of each plot were harvested and above ground dry matter was recorded after drying in oven at 75° C for 48 h. And finally,seed yield per plot divided by the total biomass (dry weight of plants plus yield) was obtained Harvest Index. Finally all data were analyzed by MSTAT-C statistical software and the means were compared by Duncan′s Multiple Range Test (DMRT) at the 5% probability level.

RESULTS AND DISCUSSION

Number of pods per plant:

Number of pods per plant was decreased significantly under water stress conditions. The highest average number of pods per plan was 23.26 from normal irrigation (I_0) and the lowest average was 21.23 from drought stress (I_1) (Table 1). Acosta-Gallegos and Adams [1] reported that water deficit reduced the number of flower buds, and Flower abortion is occurs.finally number of pods per plant reduced. Kumar et al [31] observed that drought, decreased number of pods per plant of Pea. Many studies similar to our experiment, reported that water stress reduces pods number per plant [19,42,48].Proline application (soaking and spraying) was significant effect on the number of pods per plant and it increased.The highest no. pods plant^{-1} plant was 25.37 from spraying of proline in two stages of 6-leafy and flowering in proline concentration of 20 mM (P_4) and the lowest no. pods plant^{-1} plant was 19.76 from no use of proline (P_0) (Table 1). Aspinal and Paleg [7] observed that proline application (spraying), increased yield components under drought stress. Interaction effects of water stress and proline application on number of pods per plant was significant. The drought has reduced the number of pods. But the seed soaking treatment in proline concentration of 60 mM, the number of pods increased. Also the the number of pods per plant in the absence of proline application fell sharply due to drought stress. But in proline application, this decrease was little. The highest no. pods plant^{-1} (25.75) was obtained in the combination treatment of irrigation after 50 mm evaporation and spraying of proline in two stages of 6-leafy and flowering in proline concentration of 20 mM (I_0P_4) and the lowest no. pods plant^{-1} (16.06) was produced in the combination treatment of irrigation after 100 mm evaporation and no use of proline (I_1P_0) (Table 2). In water stress conditions, the maximum no. pods plant^{-1} was observed in spraying of proline in two stages of 6-leafy and flowering in proline concentration of 20 mM(P_4) (Table 2).

Number of seeds per pod:

Number of seeds per pod was decreased significantly under water stress conditions. The highest average number of seeds per pod was 8.77 from normal irrigation (I_0) and the lowest average was 8.27 from drought stress (I_1) (Table 1). 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 [14,16,21]. In summary, prevailing drought reduces plant growth and development, leading to hampered flower production and grain filling and thus smaller and fewer grains.Many studies similar to our experiment, reported that water stress reduces seeds number per pod [42] of common bean.Proline application (soaking and spraying) was significant effect on the number of seeds per pod and it increased.The highest no. seeds pod^{-1}plant was 9.01 from seed soaking for 12h in proline concentration of 20 mM(P_1) and the lowest no. seeds pod^{-1}plant was 8. 09 from no use of proline (P_0) (Table 1). Aspinal and Paleg [7] observed that proline application, increased yield components especially number of seeds per pod under drought stress. The highest no. seeds pod^{-1} (9.15) was obtained in the combination treatment of irrigation after 50 mm evaporation and seed soaking for 12h in proline concentration of 60 mM (I_0P_3) and the lowest no. seeds pod^{-1} (8) was produced in the combination treatment of irrigation after 100 mm evaporation and no use of proline (I_1P_0) (Table 2). In water stress conditions, the highest no. seeds pod^{-1} was observed in seed soaking for 12h in proline concentration of 20 mM(P_1) (Table 2).

1000-Seeds weight:

1000-seeds weight was decreased significantly under water stress conditions. The highest average 1000-seeds weight was 215.88 gr from normal irrigation (I_0) and the lowest average was 210.57 gr from drought stress (I_1) (Table 1). Virk et al [50] observed that the reduction in photosynthetic materials and reduction in material transfer and also reduce during grain filling of the seed weight decreases in drought stress. A reduction in grain filling occurs due to a reduction in the assimilate partitioning and activities of sucrose and starch synthesis enzymes [3]. Many studies similar to our experiment, reported that water stress reduces seed weight [42,48] of common bean.Proline application (soaking and spraying) was significant effect on the 1000-seeds weight and it increased.The highest 1000-seeds weight was 227.88 gr from spraying of proline in two stages of 6-leafy and flowering in proline concentration of 40 mM (P_5) and the lowest 1000-seeds weight was 199.3 gr from no use of proline (P_0) (Table 1). Walton et al [51] reported that the use of proline and other amino acids in plant lead to increased seed weight under drought stress. Gupta and Das [22] believe that proline and other amino acids with nitrogen and carbon storage in the plant will increase grain weight and yield. The highest 1000-seeds weight (230.798 gr) was obtained in the combination treatment of irrigation after 50 mm evaporation and spraying of proline in two stages of 6-leafy and flowering in proline concentration of 40 mM (I_0P_5) and the lowest 1000-Seeds weight (194.27 gr) was produced in the combination treatment of irrigation after 100 mm evaporation and no use of proline (I_1P_0) (Table 2). In water stress conditions, the maximum 1000-seeds weight was obtained in spraying of proline in two stages of 6-leafy and flowering in proline concentration of 40 mM(P_5) (Table 2).

Seed yield:

Seed yield was decreased significantly under water stress conditions. The highest average seed yield was 880.96 g m^{-2} from normal irrigation (I_0) and the lowest average was 744.46 g m^{-2}from drought stress (I_1) (Table 1). Pannu and Singh [40] reported that grain yield reduction due to drought stress,owing to the negative effects of stress on leaf area, photosynthesis, vegetation, crop growth rate and yield component.This results with the findings of kumar et al [31] corresponded About Reduced cowpea yield. Many studies similar to our experiment, reported that water stress reduces seed yield [18, 42, 48].Proline application (soaking and spraying) was significant effect on the seed yield and it increased.The highest seed yield was 929.68 g m^{-2}from spraying of proline in two stages of 6-leafy and flowering in proline concentration of 20 mM (P_4) and the lowest seed yield was 637.76 g m^{-2}from no use of proline (P_0) (Table 1). The different results indicate that external application of proline in the range of 10 to 250 mM leads to increased yield under water stress [2]. Aspinal and Paleg [7] found that the accumulation of proline and other amino acids in plants, thereby increasing the cells ability to absorb water. And by increased grain weight, yield also increased. Stewart and Voetberg [47] reported that the proline application at a concentration of 0.1 mM lead to increased grain yield under drought stress relative to the control condition (no spray) in barley.

The highest seed yield (1008.96 g m^{-2}) was obtained in the combination treatment of irrigation after 50 mm evaporation and spraying of proline in two stages of 6-leafy and flowering in proline concentration of 20 mM (I_0P_4) and the lowest seed yield (489.88 g m^{-2}) was produced in the combination treatment of irrigation after 100 mm evaporation and no use of proline (I_1P_0). (Table 2). In water stress conditions, the highest seed yield was recorded in spraying of proline in two stages of 6-leafy and flowering in proline concentration of 40 mM(P_5) which was statistically similar to spraying of proline in two stages of 6-leafy and flowering in proline concentration of 20 mM(P_4) (Table 2).

Biomass:

Biomass was decreased significantly under water stress conditions. The highest average biomass was 1858.3 g m^{-2} from normal irrigation (I_0) and the lowest average was 1743.87 g $m^{-2}$from drought stress (I_1) (Table 3). Investigation of Major [34] on a dry matter accumulation has shown that drought stress reduced plant dry weight due to reduced photosynthetic materials.These results with the findings Anyia and Herzog [4] about reduced the biomass of cowpea under drought stress corresponded. These results are in line with those of [20, 23,45] who observed that water stress decreased biomass production corresponded.Proline application (soaking and spraying) was significant effect on the biomass and it increased.The highest biomass was 2006.29 g $m^{-2}$from spraying of proline in two stages of 6-leafy and flowering in proline concentration of 40 mM (P_5) and the lowest biomass was 1572.92 g $m^{-2}$from no use of proline (P_0) (Table 3). Ashraf and Foolad [5] also reported that application of proline (foliar spray) increased leaf area and dry matter accumulation in sorghum.we observed using foliar proline even in low water, plants growth is less affected by the drought stress. Finally, the application of proline, synthesis pathway taken several steps ahead and spend the extra carbon for growth and production.Proline application with determine concentration can increase the growth of plant.As result proline also lead to increase biomass plant.The highest biomass (2061.5 g m^{-2}) was obtained in the combination treatment of irrigation after 50 mm evaporation and spraying of proline in two stages of 6-leafy and flowering in proline concentration of 40 mM (I_0P_5) and the lowest biomass (1404.41 g m^{-2}) was produced in the combination treatment of irrigation after 100 mm evaporation and no use of proline (I_1P_0) (Table 4). In water stress conditions, the highest biomass was recorded in spraying of proline in two stages of 6-leafy and flowering in proline concentration of 40 mM(P_5) which was statistically similar to spraying of proline in two stages of 6-leafy and flowering in proline concentration of 20 mM(P_4) (Table 4).

Harvest Index (HI):

Harvest index was decreased significantly under water stress conditions. The highest average harvest index was 32.11 % from normal irrigation (I_0) and the lowest average was 29.83 % from drought stress (I_1) (Table 3). Terana and Singh [49] reported that the low-water conditions lead to reduction of bean pod and finally harvest index decreased. In our experiment, Due to the decreased number of pods, number of seeds per pod and 1000 seed weight under water stress, harvest index decreased.Proline application (soaking and spraying) was significant effect on the harvest index and it increased.The highest harvest index was 33.39% from seed soaking for 12h in proline concentration of 20 mM(P_1) and the lowest harvest index was 28.85% from no use of proline (P_0) (Table 3). In this experiment, we observed that proline application (spraying and soaking) due to increased yield and yield component,finally lead to increased harvest index. Stewart and Voetberg [47] reported that the use of proline and other amino acids, lead to increased harvest index. The highest harvest index (33.83 %) was obtained in the combination treatment of irrigation after 50 mm evaporation and seed soaking for 12h in proline concentration of 20 mM (I_0P_1) and the lowest harvest index (26.82 %) was produced in the combination treatment of irrigation after 100 mm evaporation and no use of proline (I_1P_0) (Table 4). In water stress conditions, the highest harvest index was recorded in seed soaking for 12h in proline concentration of 20 mM (P_1) (Table 4).

Plant Height:

Plant height was decreased significantly under water stress conditions. The highest average plant height was 57.27 cm from normal irrigation (I_0) and the lowest average was 55.42 cm from drought stress (I_1) (Table 3). Under severe water deficiency, cell elongation of higher plants can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells [38]. Drought caused impaired mitosis; cell elongation and expansion resulted in reduced growth and yield traits [27]. Similar to our results Bideshki and Arvin [10] reported that, drought stress decreased plant height.Proline application (soaking and spraying) was significant effect on the plant height and it increased.The highest plant height was 57.99 cm from spraying of proline in two stages of 6-leafy and flowering in proline concentration of 20 mM (P_4) and the lowest plant height was 54.32 cm from no use of proline (P_0) (Table 3). Stewart and Voetberg [47] reported that application of proline and other amino acids, lead to increased plant height. Aspinal and Paleg [7] found that proline application in concentration of 0.5 nano molar cause to increased plant height. Chaves et al [14] observed Corn seed treatment with glycine betaine and proline improves emergence and seedling height and dry matter under drought stress [15]. The highest plant height (58.62 cm) was obtained in the combination treatment of irrigation after 50 mm evaporation and spraying of proline in two stages of 6-leafy and flowering in proline concentration of 40 mM (I_0P_5) and the lowest plant height (52.89 cm) was produced in the combination treatment of irrigation after 100 mm evaporation and no use of proline (I_1P_0) (Table 4). In water stress conditions, the highest plant height was

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recorded in spraying of proline in two stages of 6-leafy and flowering in proline concentration of 20 mM(P_4)(Table 4).

Conclusion:

The results in our experiment showed that water stress, decreased yield and yield components, biomass, harvest index and plant height. Proline application had a positive impact on the measured traits under normal and water stress condition. The highest seed yield (1008.96 g m^{-2}) was obtained in the combination treatment of irrigation after 50 mm evaporation and spraying of proline in two stages of 6-leafy and flowering in proline concentration of 20 mM (I0P4) and the lowest seed yield (489.88 g m^{-2}) was produced in the combination treatment of irrigation after 100 mm evaporation and no use of proline (I1P0). The results showed that the proline applied at different levels, especially as foliar spraying at two stages of 6-leafy and flowering cause more production of cowpea under normal and water stress conditions.The present investigation suggests that application of proline may help decrease the adverse effects of drought in cow pea.

Table 1: Effect of water stress and Proline application on yield and yield components of cow pea.

Treatments	No. pods $plant^{-1}$	No. seeds pod^{-1}	1000-Seeds weight (g)	Seed yield (g m^{-2})
Irrigation				
Control	23.26 a	8.77 a	215.88 a	880.96 a
Water stress	21.24 b	8.27 b	210.57 b	744.46 b
proline application (soaking and spraying)				
P0	19.76 d	8.09 b	199.3 c	637.76 d
P1	22.51 bc	9.01 a	207.01 bc	839.61 bc
P2	21.75 bc	8.49 ab	210.17 b	783.04 c
P3	20.82 cd	8.61 ab	215.48 b	772.77 c
P4	25.37 a	8.43 b	216.46 b	929.68 a
P5	23.15 b	8.57 ab	227.88 a	905.89 ab
P6	22.4 bc	8.45 b	215.74 b	820.22 c

Means with the same letter in each column and treatment are not significantly different at probability level of 5% using DMRT. (p_0) : no use of proline, (P_1): cow pea seeds were soaked for 12h in proline concentration of 20 mM, (P_2) : cow pea seeds were soaked for 12h in proline concentration of 40 mM, (P_3) : cow pea seeds were soaked for 12h in proline concentration of 60 mM, (P_4) : spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 20 mM, (P_5) : spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 40 mM, (P_6) : spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 60 mM.

Table 2: Interaction effects of water stress and Proline application on yield and yield components

Irrigation	proline application (mM)	No. pods $plant^{-1}$	No. seeds pod^{-1}	1000-Seeds weight (g)	Seed yield (g m^{-2})
	P0	23.45 abc	8.19 bcd	204.32 cd	776.64 def
	P1	23.08 bcd	9.05 a	209.33 bc	873.56 bcd
	P2	22.95 bcd	8.73 abcd	211.2 bc	846.22 bcde
Control	P3	20.31 e	9.15 a	216.19 abc	805.68 cdef
	P4	25.75 a	8.86 abc	221.71 ab	1008.96 a
	P5	23.75 abc	8.66 abcd	230.98 a	953.3 ab
	P6	23.56 abc	8.79 abcd	217.48 abc	902.35 abc
	P0	16.06 f	8 d	194.27 d	498.88 g
	P1	21.93 cde	8.97 ab	204.7 cd	805.65 cdef
	P2	20.56 de	8.25 bcd	210.22 bc	719.86 f
Water stress	P3	21.33 cde	8.08 cd	214.78 bc	739.85 ef
	P4	25 ab	8.01 d	211.21 bc	850.39 bcde
	P5	22.54 bcde	8.01 d	224.79 ab	858.46 bcde
	P6	21.25 cde	8.11 cd	214.01 bc	738.08 ef

Means with the same letter in each column are not significantly different at probability level of 5% using DMRT (p_0) : no use of proline, (P_1): cow pea seeds were soaked for 12h in proline concentration of 20 mM, (P_2) : cow pea seeds were soaked for 12h in proline concentration of 40 mM, (P_3) : cow pea seeds were soaked for 12h in proline concentration of 60 mM, (P_4) : spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 20 mM, (P_5) : spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 40 mM, (P_6) : spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 60 mM.

Table 3: Effect of water stress and Proline application on biomass, harvest index and plant height of cow pea.

Treatments	Biomass (g m^{-2})	harvest index %	plant height (cm)
Irrigation			
Control	1858.3 a	32.11 a	57.27 a
Water stress	1743.87 b	29.83 b	55.42 b
proline application (soaking and spraying)			
P0	1572.92 d	28.85 c	54.32 d
P1	1675 cd	33.39 a	56.77 ab
P2	1795.96 c	30.2 bc	55.82 bc
P3	1776.04 c	30.26 bc	55.33 cd
P4	1964.96 ab	32.05 ab	57.99 a
P5	2006.29 a	31.05 abc	57.97 a
P6	1816.42 bc	30.99 abc	56.21 bc

Means with the same letter in each column and treatment are not significantly different at probability level of 5% using DMRT. (p_0) : no use of proline, (P_1): cow pea seeds were soaked for 12h in proline concentration of 20 mM, (P_2) : cow pea seeds were soaked for 12h in proline concentration of 40 mM, (P_3) : cow pea seeds were soaked for 12h in proline concentration of 60 mM, (P_4) : spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 20 mM, (P_5) : spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 40 mM, (P_6) : spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 60 mM.

Table 4: Interaction effects of water stress and Proline application on biomass, harvest index, plant height

Irrigation	proline application (mM)	Biomass (g m^{-2})	harvest index %	plant height (cm)
Control	P0	1741.41 cd	30.88 abcd	55.75 cde
	P1	1707.5 cd	33.83 a	57.6 abc
	P2	1811.33 bcd	31.84 abc	56.08 bcde
	P3	1818.75 bcd	30.63 abcd	57.37 abcd
	P4	2028.08 ab	33.2 ab	58.14 a
	P5	2061.5 a	31.54 abc	58.62 a
	P6	1838.05 abcd	32.85 abc	57.31 abcd
Water stress	P0	1404.41 e	26.82 d	52.89 f
	P1	1642.5 d	32.95 ab	55.93 bcde
	P2	1780.58 cd	28.56 cd	55.56 de
	P3	1733.33 cd	29.9 abcd	53.29 f
	P4	1901.83 abc	30.9 abcd	57.85 ab
	P5	1951.08 abc	30.56 abcd	57.33 abcd
	P6	1793.33 bcd	29.12 bcd	55.12 e

Means with the same letter in each column are not significantly different at probability level of 5% using DMRT (p_0) : no use of proline, (P_1): cow pea seeds were soaked for 12h in proline concentration of 20 mM, (P_2) : cow pea seeds were soaked for 12h in proline concentration of 40 mM, (P_3) : cow pea seeds were soaked for 12h in proline concentration of 60 mM, (P_4) : spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 20 mM, (P_5) : spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 40 mM, (P_6) : spraying on plant leaves in two stages of 6-leafy and flowering in proline concentration of 60 mM..

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