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THE INFLUENCE OF SPRAYING OF PROLINE ACID ON SOME GROWTH CHARACTERISTICS OF COWPEAS PLANT (*VIGNA UNGUICULATA* L.) SUBJECTED TO DROUGHT STRESS

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Abstract

The experiment was conducted for the 2018-2019 growth season. (0, 20, 40, 60) mg/L^{-1} in some growth characteristics of the drought stress *Vigna unguiculata* L. plant with three periods (4, 8, 12) days. The experiment is designed by R.C.B.D. (Randomized Complete Blocks Design) with three replicates per treatment. The mean was compared with the use of the least significant difference at the level of probability (0.05), and the following results were shown

1. The effect of proline acid in its different concentrations resulted in a significant increase in all the studied characteristics, which exceeded the concentration of 0 mg.L^{-1} of proline in percentage of sodium, and the concentration of 20 mg.L^{-1} of proline in the height of the plant, and the concentration of 40 mg. 1 of proline in both root length, protein percentage, total chlorophyll content in leaves, and a concentration of 60 mg/L^{-1} of proline in both calcium percentage, salicylic acid concentration.
2. The results showed that the effect of drought stress on irrigation periods with the spacing of 4 to 12 days resulted in a significant decrease in some growth characteristics of the plant (plant height, root length, calcium percentage, protein percentage, total chlorophyll content), except sodium and salicylic acid concentration. When exposed to a longer stress period at 12 days.
3. The effect of overlap between the study agents was significant in removing the negative effect of drought stress, where the concentration was 40 mg/L^{-1} Proline has a role in removing the negative effect of drought stress of period 4 Days in plant height, root length, calcium percentage, 8-day protein percentage, total chlorophyll content and 60 mg/L^{-1} concentration of proline and the same period in the salicylic acid concentration, at a concentration of 0 mg L^{-1} of 12-day proline acid in sodium percentage.

Keywords **:** Proline Acid, Growth Characteristics, *Vigna unguiculata* L., Drought Stress

Introduction

The Leguminosae family is a large family with 35 genera and about 300 species, divided into three separate aggregates but some sources including the Iraqi botanical encyclopedia are divided into three separate families, Mimosoideae, Papilionoideae and Caesalpinioideae (Al-Mousawi,1987). The family of legumes is an important food source and its importance lies in its ability to install air nitrogen through bacterial nodes that are at its roots, since legumes improve soil properties, and a section of internal proteins is more intensify than the outer layers and tolerant a longer drought period because they have deep roots (Moghazy *et al.*, 2014).

Vigna unguiculata L. of important legumes whose grain is mainly used as human food, while the total of the vegetables contain after it has been harvested high and desired nutritional value of the livestock and are essential crops in crop systems because they are fast-growing and protect the soil from erosion and have a great ability to stabilization the atmospheric nitrogen through bacterial nodes that have their soil, which increases soil fertility (Singh *et al.*, 2002). cowpea is a food-rich crop with 86% of water, 3.3% of proteins, 5.9% of carbohydrates, and 44 calories can be obtained fresh green, stored in cans or cooked in other different ways (Hassan, 1997). There has been insufficient attention from researchers in Iraq, particularly since research on the development of new varieties is almost non-existent, although the crop has been cultivated in considerable areas in the central and southern regions (Burton and Brownie, 2006).

It is Proline The most important amino acids involved in protein formation, Proline whereas the structure of cyclic amino acid can be found in plants freely and metabolizes of the glutamic acid by the enzyme Pyrroline-5-carboxylate synthetase within the cell specifically in cytosol cell A Proline has the ability to bind to other amino acids through the NH_2 group which is found in its composition and through it will lead to the formation of proteins that share the cellular membrane construction of the cell with portability high tolerance to environmental stresses such as drought and other stresses also exists freely in the mitochondria to link with some of the amino acids and the most important of these acids is the amino acid Arginine This association occurs especially when exposed to stresses (Szabados and Savouré, 2010). The amino acid proline is an antioxidant by its contribution to the elimination of effective O_2 groups and prevents the accumulation of malonday aldehyde, which accumulates as a result of oxidative stress, maintaining of cellular membranes and no oxidation of lipids metabolism (Soshinkova *et al.*, 2013). The accumulation of proline within the plant is usually as a reaction to their adaptation or sensitivity to particular stress (low temperatures, salinity or lack of water) that can be known early in the plant life cycle. (Bates *et al.*, 1973) Proline is present in many plants, especially in the cowpea plant, indicates that their accumulation is independent of the growth stage of the plant and is related to water nutrition. It plays an important role at the cellular level in maintaining high internal osmosis pressure. Some plants adapt to harsh environmental conditions such as drought, high salinity through the accumulation of some free amino acids, especially proline amino acid, which has an important role in regulating the osmosis cell cytoplasm as complementary solubility (Bartels and Sunkar, 2005). During this osmosis effort, the

combination of proline increases the proportion of NADPH, NADP+ intervention in cell metabolism, so it plays a role in aerobic respiration as a source of energy so it helps the plant to disposal stress and return to normal, as well as being convinced of the free radicals oxidized (Hare and Cress, 2004). The oxidative stress caused by damage caused by dehydration, high salinity, radiation, and diseases that lead to the production of free radicals oxidized as the product of metabolic processes that occur within a plant and of the most important ones are Superoxide, hydrogen peroxide, hydroxyl radical, as well as individual oxygen radicals, that negatively affect the portion of the existing fat in the plasma membrane that causes its permeability and thus affect the plasmid-negative effect on permeability, the plasma membrane, It also affects nuclear acids and protein building (Gratao *et al.*, 2005).

Drought: A natural climatic phenomenon that occurs largely in most cities of the world and that has been expressed by Vannozzi and others (Vannozzi, *et al.*, 1999) as a situation in which water availability scarce to point so that the plant cannot absorb water as it is in its natural position to match evaporation - transpiration, and, in other words, drought means that the drying of readily accessible water from the root zone is equivalent to the water voltage of the plant to the water voltage of the soil and at that The plant reaches the permanent wilt point. The problem of drought is a global problem that does not specify a particular country because of the large demand for water use as a result of the increase of large populations in cities. In this way, plans should be made for proper water investment, especially in the agricultural sector, which is considered to be the biggest water consumer in comparison with many other sectors, and through this research we are working to find appropriate solutions in water consumption through the use of materials that make the plant more tolerant to the drought and consume less water than the water required by its natural state, this will lead to the provision of large amounts of water and increase the cultivated areas. This research aims, therefore, at:-

1. To study the effect of spraying with proline acid in raising water use efficiency to preserve and not wastewater, and to solve the drought problem that Iraq is currently experiencing through the use of low-cost materials (proline acid) to make plants more drought-tolerant for a longer period, and make it consumes water in quantities less, and this will lead to the provision of large amounts of water, and thus eliminating another problem, desertification, by increasing agriculture of large green areas.
2. Find the best treatment for concentrations of proline acid used in resistance drought stress.
3. A statement of the effect of proline amino acid on some growth characteristics of the plant exposed to the stress of drought and its overlap with drought stress.

Materials and Methods

The experiment was carried out during the season 2018-2019 plants cultivation was in SANDIN (pot) The experiment included the study of the effect of spraying the amino acid proline on some growth characteristics of the plant and its role in raising water efficiency by making the plant tolerant the drought for a longer period, the spray will be of acid with four concentrations, respectively (0, 20, 40, 60, 60) mg /L^{-1}, The drought stress will be the periods in an irrigation blocking of (Yassin, 1992) :

1- irrigation every 4 days (Treatment of Control).

2- irrigation every 8 days.

3- irrigation every 12 days.

The experiment was designed according to the Randomize Complete Block Design (RCBD) and three replicates as the experiment included 36 experimental units, with one replicate, contains 12 experimental units, the total number of experimental units is 36 experimental units (number of the pot) is 36 pot each pot capacity of 5kg/soil. A sample of soil was taken before Agriculture for physical and chemical analysis, as shown in Table (1).

Table 1 : Some physical and chemical characteristics of the soil of the experiment before Agriculture

Elements		Value	Unit
Soil Texture		Mixture	-----
Soil separators	Sand	290	g.kg^{-1} soil
	Silts	410	
	Clay	240	
Reaction Degree (pH)		7.1	-----
Electrical conductivity (EC)		3.20	Dissemens.M^{-1}
ready Nitrogen		40.37	Mg.kg^{-1} soil
ready Phosphorus		11.90	Mg.kg^{-1} soil
ready Potassium		218	Mg.kg^{-1} soil

The NPK, 20=20=20, was added to all experimental units before agriculture at a rate of 0.50 g/pot, then seeds were planted on 26/8/2018 with nine seeds per pot, according to the experiment, and then dried to six seeds a week after planting, and all crop service operations were performed from irrigation, rupture and disrepair as needed until the end of the experiment. In the early morning on 2/10/2018, the plants were sprayed on the leaves when their number became 3-4 leaves 37 days after the date of planting, using a 2 liter hand sprinkler, taking samples from the plant's vegetable portion at the rate of three plants from each experimental unit to study characteristics some plant and As follows:

1- Plant height (cm) : The plant height was measured for three random plants each experimental unit by the ruler.

2- Root length (cm) : The root length of three random plants for each experimental unit was measured from its point of contact with the stem to the end of the root by a ruler's.

3- Calcium percentage estimate (%) in the vegetable total : The crushed dried samples were digested according to the method (Gresser and Parson, 1979) the percentage of calcium in digested samples in the vegetable total was estimated using the Atomic absorption spectrophotometer and according to the method (Wimberly, 1968).

4- Sodium percentage estimate (%) in the vegetable total : the percentage of sodium is estimated by the Flame photometer method and by the method (Page *et al*, 1982).

5- Protein percentage estimate (%) in the vegetable total : The percentage of protein in the vegetable total

is estimated by multiplying the nitrogen ratio by a fixed factor (6.25) according to the method (Vopyan, 1984)

Protein percentage = N% x 6.25

6- **Estimate of the total chlorophyll content in leaves ($mg.g^{-1}$) :** Total chlorophyll content estimated by the method (Goodwin, 1976)

7- **Salicylic acid concentration estimate ($\mu g.mL^{-1}$) :** Estimated by the method (Warrier *et al*, 2013). The results were statistically analyzed according to the design applied and adopted the least significant difference at the level of probability 0.05 (SAS, 2012).

Results and Discussion

Plant height (cm)

The results of Table 2 indicate a significant difference between irrigation periods treatments in the mean plant height. The irrigation treatment every 4 days achieved the highest mean of 20.9 cm while the irrigation treatment achieved every 12 days less mean of 15.4 cm. The reason for the superiority of irrigated plants every 4 days is that the physiological and metabolic processes in the plant, including cell division, elongation and other were not affected and were normal in this period of irrigation and this treatment was superior, the spacing in the irrigation periods every 8 and 12 days to a significant decrease in the height of the plant that this The decline led to lack of water in the plant and its role in many of physiological processes in the plant, including reduced cell division, loss of spell pressure and reduced cell elongation, which reduced plant height when exposed to longer drought periods and as well as caused to defect in plant internal hormonal system, which leads to an accumulation of the basic acid in the plant, reducing cell division and reducing its size in apical regions of the plant, including the stems tops (Al-Shahat, 2000; Fanaei *et al.*, 2015) and also caused severe stress to the lack of the main elements of the building of primary and secondary metabolism of the plant resulting in a decrease in proteins and weak plant growth and reduced rates.

The results of Table 2 show that the sprayed the cowpea plants with a concentration of 20 mg L^{-1} of proline significantly superiority and achieved the highest mean to the plant height of 21.1 cm and a significant difference from all other treatments, especially the treatment of control (0 mg L^{-1}), which gave less mean of 13.6 cm, The reason for the superiority of plants sprayed with a concentration of 20 mg L^{-1} of proline acid to the positive role in regulating the Osmotic potential by regulating the pressure and water potential, which increases the cell's ability to draw water from the center of growth and then increase the growth of the plant and sustain the elongation of cells and sustainability open stents and process photosynthesis (Yassin, 1992).

The effect of the overlap between the two agents was significant in the plant height (Table 2). The irrigated plants were given every 4 days and the concentration was 40 mg / L^{-1} of proline. The highest to overlap mean was 25.0 cm and did not differ significantly from the irrigated plants every 8 days and sprayed with a concentration of 20 mg L^{-1} of the proline, which gave 23.3 cm, while irrigated plants gave every 12 days and without the proline spray, less means to overlap was 12.2 cm. Proline proved by 20 and 40 mg/L^{-1}, its ability to reduce the negative effects of water stress through Its active role in increasing the regulation of Osmotic potential through the proline aggregation in the cell plant under stress conditions resulting in a significant increase in plant height (Mohanty and Matysik, 2001).

Table 2 : The effect of proline acid in plant height (cm) to plant cowpea subjected to drought stress

Mean	Proline Concentrations (mg / L^{-1})				irrigation Periods (Day)
	60	**40**	**20**	0	
20.9	21.0	25.0	22.3	15.3	irrigation every 4 days
18.2	18.7	17.3	23.3	13.3	irrigation every 8 days
15.4	15.7	16.3	17.6	12.2	irrigation every 12 days
1.3	2.6				L.S.D.0.05
	18.4	19.5	21.1	13.6	Mean
	1.5				L.S.D.0.05

Root length (cm)

The results of Table 3 indicate a significant difference between irrigation periods treatments in the mean the root length. The irrigation treatment every 8 days achieved the highest mean of 5.39 cm while the irrigation treatment achieved every 12 days less mean of 4.59 cm. The reason for the superiority of irrigated plants every 8 days is that the physiological and metabolic processes in the plant, including cell division, elongation and other were not affected and were normal in this period of irrigation and this treatment was superior, the spacing in the irrigation period every 12 days to a significant decrease in the root length that this The decline led to a shortage of water in the plant and its role in many of physiological processes in the plant, including reduced cell division, and reduced cell elongation, and reduced of root length (Table 3) and as well as caused to defect in plant internal hormonal system, which leads to an accumulation of the basic acid in the plant,, reducing cell division and reducing its size in apical regions of the plant, including the roots tops (Al Shahat, 2000; Fanaei *et al.*, 2015). It is believed that water stress leads to a decrease in auxin content due to IAA Oxidase enzyme activity, which prevents the descent of Auxin and the associated agents of the leaves, resulting in weakening plant rooting (Shaheed, 2013).

The results of Table 3 show that the sprayed the cowpea plants with a concentration of 40 mg L^{-1} of proline significantly superiority and achieved the highest mean to the Root length of 5.93 cm. While the control treatment (0 mg L^{-1}) achieved less mean of 4.00 cm due to a concentration superiority 40 mg L^{-1} from proline to the important role of proline in increasing plant growth and resistance (Gardener and Gillman, 2001). In addition to its role in providing the plant with the necessary energy to carry out important vital activities, including growth in the root total (Tonon *et al.*, 2004). The effect of the overlap between the two study agents was significant in the root length (Table 3) Also, Proline acid with concentrations 40 and 60 mg L^{-1} proved its ability to reduce the negative effects of water stress through its active role in increasing the regulation of the Osmotic potential through of proline aggregation in the plant cells under stress conditions, resulting in a significant increase in root length (Mohanty and Matysik, 2001). The irrigated plants were given every 4 days and with concentration was 40 mg/L^{-1} of proline. The highest to overlap mean was 6.40 cm and did not differ significantly from the irrigated plants

every 8 days and sprayed with concentrations of 40 and 60 mg L^{-1} of proline which gave 6.17 and 6.07 cm sequentially and overlapping was significant. The concentration of 60 mg / L^{-1} of proline reduced the negative effect of drought stress in 12 days. The root length was 5.67 cm While the irrigated plants were given every 12 days without spray the proline with a less to overlap mean of 3.20 cm.

Table 3: The effect of proline acid in root length (cm) to plant cowpea subjected to drought stress

Mean	Proline Concentrations (mg / L^{-1})				irrigation Periods (Day)
	60	40	20	0	
5.14	4.13	6.40	5.53	4.50	irrigation every 4 days
5.39	6.07	6.17	5.03	4.30	irrigation every 8 days
4.59	5.67	5.23	4.25	3.20	irrigation every 12 days
0.62	1.25				L.S.D.0.05
	5.29	5.93	4.94	4.00	Mean
	0.72				L.S.D.0.05

Calcium percentage in the vegetable total (%)

The results of Table 4 indicate a significant difference between irrigation periods treatments in the mean Calcium percentage, the irrigation treatment every 4 days achieved the highest mean of 1.51 % while the irrigation treatment achieved every 12 days less mean of 1.17%. Decreased ratio of a significant decreased at a spacing in the irrigation period from 4 days to 12 days. This is due to the superiority of irrigated plants every 4 days to its superiority in the root length through increasing root growth (Table 3), which was positively reflected in the increase in calcium percentage through increasing the absorption of nutrients by the roots.

The results of Table 4 show that the sprayed the cowpea plants with a concentration of 60 mg L^{-1} of proline significantly superiority and achieved the highest mean to calcium percentage of 1.56 % and a significant difference from all other treatments, especially the treatment of control (0 mg L^{-1}), which gave less mean of 0.99 %, This is due to a concentration superiority 60 mg L^{-1} from proline to Its superiority in the root length through increasing root growth (Table 3), which was positively reflected in the increase in calcium percentage through increasing the absorption of nutrients by the roots.

The effect of the overlap between the two study agents was significant in calcium percentage (Table 4) Also, Proline acid with concentrations 40 and 60 mg L^{-1} proved its ability to remove the negative effects of water stress resulting in an increase in calcium percentage, The irrigated plants were given every 4 days and with concentration was 40 mg/L^{-1} of proline. The highest to overlap mean was 1.81 % and did not differ significantly from the irrigated plants every 4 and 8 days and sprayed with concentration of 60 mg L^{-1} of proline which gave 1.69 and 1.61 % sequentially, the reason for its superiority in root length is due to increased root growth (Table 3) which was positively reflected in the increase in calcium percentage through increasing the absorption of nutrients by the roots. The increase in calcium percentage is due to the role of proline acid in removing the negative effect of drought stress, improving plant growth and increasing calcium absorption , This is confirmed by the results of the interference between proline acid and drought stress, which showed the role of proline in removing the negative effect of drought stress, while irrigated plants were given every 12 days and without spraying the proline less to overlap mean of 0.96%.

Table 4: The effect of proline acid in Calcium percentage in the vegetable total (%) to plant cowpea subjected to drought stress

Mean	Proline Concentrations (mg / L^{-1})				irrigation Periods (Day)
	60	40	20	0	
1.51	1.69	1.81	1.52	1.03	irrigation every 4 days
1.29	1.61	1.25	1.35	0.97	irrigation every 8 days
1.17	1.36	1.16	1.22	0.96	irrigation every 12 days
0.11	0.22				L.S.D.0.05
	1.56	1.41	1.36	0.99	Mean
	0.13				L.S.D.0.05

Sodium percentage in the vegetable total (%)

The results of Table 5 indicate a significant difference between irrigation periods treatments in the mean sodium percentage, the irrigation treatment every 12 days achieved the highest mean of 0.162% while the irrigation treatment achieved every 4 days less mean of 0.137%. The increase in the dry periods increases the percentage of sodium when drought exposure for a longer period, which may be due to its effect on the cell's Osmotic potential, as well as the role of its negative ions in the cell membrane as it replaces the calcium ions that gave the less mean dry 12 days (Table 4) Causing a decrease in permeability of the membrane and this leads to loss of integrity and lead to increased accumulation of sodium ions and decrease the concentration of ions necessary for growth.

The results of Table 5 show that the non- sprayed the cowpea plants with proline acid of (0 mg L^{-1}) significantly superiority and It gave the highest mean to sodium percentage of 0.187%. While the treatment (40 mg L^{-1}) of proline It gave less mean of 0.120 %, The reason for this decrease in the percentage of sodium when spraying with the proline acid is due to its positive role in reducing the sodium effect in the plant where spray of proline helped the plant to adapt for drought stress by reserving sodium ions in gaps called saline glands in root and stem cells and activating the movement of potassium ion to replace it, which confirms the positive role of proline in reducing the harmful effect of drought stress.

The effect of the overlap between the two study agents was significant in sodium percentage (Table 5) which confirms the role of proline acid in reducing the negative effect of drought stress since irrigated plants every 12 days and the non- sprayed (0 mg. L^{-1}) gave the highest mean of 0.201% of sodium percentage, but, irrigated plants every 4 days and when the proline was added, the sodium percentage was reduced by a concentration of (40 mg L^{-1}) of proline which gave the less mean of 0.104%, which confirms the positive role of proline in removing the negative effect of drought through reducing the sodium percentage when added.

Table 5 : The effect of proline acid in Sodium percentage in the vegetable total (%) to plant cowpea subjected to drought stress

Mean	Proline Concentrations (mg / L^{-1})				irrigation periods (Day)
	60	40	20	0	
0.137	0.156	0.104	0.122	0.164	irrigation every 4 days
0.147	0.120	0.130	0.146	0.195	irrigation every 8 days
0.162	0.147	0.125	0.176	0.201	irrigation every 12 days
0.013	0.027				L.S.D.0.05
	0.141	0.120	0.148	0.187	Mean
	0.015				L.S.D.0.05

Protein percentage in the vegetable total (%)

The results of Table 6 indicate a significant difference between irrigation periods treatments in the mean Protein percentage. The irrigation treatment every 8 days achieved the highest mean of 7.67% and did not differ significant irrigation treatment every 4 days (7.12%), while every 12day irrigation treatment achieved the less mean of 6.55 %, It has decreased by a significant reduction at the spacing of irrigation periods from 4 to 12 days. The reason for the superiority of irrigated plants every 8 days to Its superiority in the root length through increasing root growth (Table 3), which was positively reflected In increasing the percentage of plants from nitrogen and phosphorus, which are considered to be important elements in the construction of amino acids and through increasing the absorption of nutrients by the roots. The results of Table 6 show that the sprayed the cowpea plants with a concentration of 40 mg L^{-1} of proline significantly superiority and achieved the highest mean to protein percentage of 8.18 % and did not differ significant the sprayed the cowpea plants with a concentration of 60 mg L^{-1} of proline of 7.48% and with a significant difference from the control treatment (0 mg/L^{-1}) which gave the less mean of 5.91%. The reason for the superiority of plants sprayed with a concentration of 40 mg L^{-1} of proline acid because spraying with proline leads to an increase in the ratio of nitrogen and phosphorus through increasing the absorption of these nutrients by the root total In addition, the increase in protein percentage in the vegetative total is due to the positive role of proline acid as one of the amino acids inside it in protein building, The increase in its internal content is stimulating protein-building as a source of nitrogen and a storage of amino groups, where it can transition from one place to another within the cell and give amino groups to the cells they need to build the protein(Yassin, 1992). The increase in the protein percentage is associated with the increase in the internal protein content of the plant as it absorbs external proline from the leaves and transferred to the plant parts, and the external addition of the proline has encouraged the vegetative growth of the plant.

The effect of the overlap between the two study agents was significant in protein percentage (Table 6) Also, Proline acid with concentrations 40 and 60 mg L^{-1} proved its ability to removal the negative effects of water stress resulting in an increase in protein percentage, The irrigated plants were given every 8 days and with concentration was 40 mg/L^{-1} of proline The highest to overlap means was 8.90 % and did not differ significantly from the irrigated plants every 4 and 8 days and sprayed with concentrations of 40 and 60 mg L^{-1} of proline which gave 8.60 and 8.46 % sequentially , In addition, the interference has had a significant effect, with a concentration of 60 mg L^{-1} of proline in reducing the negative effect of stress drought in 12 days, where the protein percentage was 8.00%, as this did not occur a significant difference in the protein ratio as well, the reason for its superiority in root length is due to increased root growth (Table 3) which was positively reflected in the increase in nitrogen and phosphorus percentage which are considered to be important elements in the construction of amino acids and through increasing the absorption of nutrients by the roots In addition to the role of proline acid, which is a source of nitrogen, this leads to an increase in the protein percentage because nitrogen are important elements in protein building through its role in the construction of amino acids, while irrigated plants were given every 12 days and without spraying the proline less to overlap mean of 5.08 %.

Table 6: The effect of proline acid in protein percentage in the vegetable total (%) to plant cowpea subjected to drought stress

Mean	Proline Concentrations (mg / L^{-1})				irrigation periods (Day)
	60	40	20	0	
7.12	5.85	8.46	7.62	6.56	irrigation every 4 days
7.67	8.60	8.90	7.10	6.08	irrigation every 8 days
6.55	8.00	7.19	5.94	5.08	irrigation every 12 days
0.65	1.31				L.S.D.0.05
	7.48	8.18	6.89	5.91	Mean
	0.75				L.S.D.0.05

The total chlorophyll content in leaves (mg.g^{-1})

The results of Table 7 indicate a significant difference between irrigation periods treatments in the mean total chlorophyll content. The irrigation treatment every 8 days achieved the highest mean of (1.216 mg.g^{-1}) and did not differ significant irrigation treatment every 4 days (1.129 mg .g^{-1}), while every 12day irrigation treatment achieved the less mean of 1.038 mg.g $^{-1}$, It has decreased by a significant reduction at the spacing of irrigation periods from 4 to 12 days . The reason for the superiority of irrigated plants every 8 days to Its superiority in the root length through increasing root growth (Table 3), which was positively reflected in an increase in the absorption of nutrients by the roots and this has led to an increase in the total chlorophyll content through increasing the elements of great importance in the chlorophyll composition, nitrogen is a component of the pyroferin ring, which is an important compound in building the chlorophyll molecule (Karron and Maranvilla, 1994). The drought severity of 12 days led to a decrease in the leaf content of chlorophyll through affecting the glutamate compound, which is the main chlorophyll compound, as the compound is transformed under the effect of the drought stress severity into proline acid This causes a lack of entry into the biological pathways of chlorophyll manufacture (Hendry *et al.*,1987).

The results of Table 7 show that the sprayed the cowpea plants with a concentration of 40 mg L^{-1} of proline significantly superiority and achieved the highest mean to the total chlorophyll content of 1.296 mg. g $^{-1}$ and did not differ significantly the sprayed the cowpea plants with a concentration of 60 mg L^{-1} of proline of 1.187 mg.g^{-1} and with a significant difference from the control treatment (0 mg

/ L^{-1}) which gave the less mean of 0.936 mg. g^{-1} This is due to a concentration superiority 40 mg L^{-1} from proline to Its superiority in the root length through increasing root growth (Table 3), which was positively reflected in an increase in the absorption of nutrients by the roots and this has led to an increase in the total chlorophyll content through increasing the elements of great importance in the chlorophyll composition, this is due to proline has a positive role in increasing chlorophyll content in the leaves, this is due to its role in stimulating chlorophyll pigments formation and plastids pellets and has a role in maintaining the enzyme activity of the plastids(Singh *et al*,1994). In addition to its role in delaying leaf senility and increasing the leaf area of the plant and thus led to an increase in the total chlorophyll content, so leaf nutrition with proline acid increases the vegetative growth of the close relationship between photosynthesis and growth, controlling the opening and closing of stents, and increasing photosynthesis pigments and protecting them from damage (Yassin, 1992).

The effect of the overlap between the two study agents was significant in the total chlorophyll content (Table 7) also, Proline acid with concentrations 40 and 60 mg L^{-1} proved its ability to reduce the negative effects of drought stress resulting in an increase in the total chlorophyll content, The irrigated plants were given every 8 days and with concentration was 40 mg/L^{-1} of proline The highest to overlap mean was 1.409 mg .g^{-1} and did not differ significantly from the irrigated plants every 4 and 8 days and sprayed with concentrations of 40 and 60 mg L^{-1} of proline which gave 1.364 and 1.340 mg .g^{-1} sequentially , In addition, the overlap has had a significant effect, with a concentration of 60 mg L^{-1} of proline in reducing the negative effect of stress drought in 12 days, where the total chlorophyll content was 1.268 mg .g^{-1}, as this did not occur a significant difference in the total chlorophyll content as well, the reason for its superiority in root length is due to increased root growth (Table 3) which was positively reflected in an increase in the total chlorophyll content through increase in the absorption of nutrients by the roots, while irrigated plants were given every 12 days and without spraying the proline less to overlap mean of 0.805 mg .g^{-1} .

Table 7: The effect of proline acid in the total chlorophyll content in leaves (mg.g^{-1}) to plant cowpea subjected to drought stress

Mean	Proline Concentrations (mg / L^{-1})				irrigation periods (Day)
	60	40	20	0	
1.129	0.928	1.340	1.208	1.040	irrigation every 4 days
1.216	1.364	1.409	1.126	0.964	irrigation every 8 days
1.038	1.268	1.139	0.941	0.805	irrigation every 12 days
0.103	0.207				L.S.D.0.05
	1.187	1.296	1.092	0.936	Mean
	0.119				L.S.D.0.05

The concentration of salicylic acid in the plant (µg.ml^{-1})

The results of Table 8 indicate a significant difference between irrigation periods treatments in the mean Concentration of salicylic acid in the plant, The irrigation treatment every 12 days achieved the highest mean of 906 µg.ml^{-1} while the irrigation treatment achieved every 4 days less mean of 620 µg.ml^{-1}. the increase in acid is due as a reaction to plant stress and is believed to be because of its role in increasing hydration and plasticity of cellular membranes that are hardened when stress severity is increased, then this mechanism gives the plant resistance against drought stress (Hayat *et al.,* 2013). It is also believed that increased concentration of acid is a product in reaction to the accumulation of toxic free radicals when exposed to stress, so the plant resorts to produce secondary metabolic compounds, including salicylic acid of the metabolism from shekmick acid (Paula, 2016).

The results of Table 8 show that the sprayed the cowpea plants with a concentration of 60 mg L^{-1} of proline significantly superiority and achieved the highest mean to Concentration of salicylic acid in the plant of 848 µg.ml^{-1} and a significant difference from all other treatments, especially the treatment of control (0 mg L^{-1}), which gave less mean of 716 µg.ml^{-1} .

The effect of the overlap between the two study agents was significant in Concentration of salicylic acid in the plant (Table 8) also, Proline acid with concentrations 40 and 60 mg L^{-1} proved its ability to removal the negative effects of drought stress resulting in an increase in Concentration of salicylic acid in the plant, The irrigated plants were given every 8 days and with concentration was 60 mg / L^{-1} of proline The highest to overlap mean was 957 µg.ml^{-1} and did not differ significantly from the irrigated plants every 12 days and sprayed with concentrations of 40 and 60 mg L^{-1} of proline which gave 944 and 902 µg.ml^{-1} sequentially This is due to the role of proline acid in removing the negative effect of drought stress, improving plant growth and increasing concentration of acid, which is confirmed by the results of interference from proline acid and drought stress This demonstrated the role of the proline in removing the negative effect of dry stress, while irrigated plants every 4 days without spraying the proline gave the less to overlap mean of 501 µg.ml^{-1}.

Table 8: The effect of proline acid in Concentration of salicylic acid in the plant (µg.ml^{-1}) to plant cowpea subjected to drought stress

Mean	Proline Concentrations (mg / L^{-1})				irrigation periods (Day)
	60	40	20	0	
620	684	678	617	501	irrigation every 4 days
841	957	841	792	775	irrigation every 8 days
906	902	944	908	870	irrigation every 12 days
28	56				L.S.D.0.05
	848	821	772	716	Mean
	32				L.S.D.0.05

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