Effect of Drought and Salicylic Acid on Vegitive Growth and Prolin Accumulation of Two Cowpea (Vigna unguiculata L.) Plants

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Abstract:

Current study was conducted during one growing season from March to July, 2021 in local farm under field conditions via plastic pots, in Biology Department/ Faculty of Science / University of Zakho -Kurdistan Region of Iraq. The experiment comprises studying soaking two local cowpea seeds, Iraqi and American cultivar in both 250 ppm of salicylic acid and distal water for four hours separately before planting. In addition to utilizing foliar spraying of 250 ppm of salicylic acid at three stages, vegetative (4^{th} true leaf stage), starting flowering stage and without spraying as a control treatment. and the effects of drought in three levels, control with continuous irrigation, three and five days of water deficit at flowering stage, followed by continuous daily irrigation (75% from field capacity) till the harvest date of the experiment. The results showed that immersion the seeds will promote seed germination percentage, plant length, number of flowers, shoot fresh weight, roots fresh weight, roots length, shoots dry weight, roots dry weight and proline accumulation. Moreover, the growth reduced as a result of water shortage due to water stress except roots length which were increased. Proline concentrations increased by rising the period of water withholding from 3 to 5 days compared to continuous irrigated treatment (control). Meanwhile, salicylic acid stimulates the accumulation amount of proline in stressed plant leaves. The Iraqi cultivar exceed the American cultivar in: seed germination percentage, plant length, number of flowers, root fresh weight, root length and roots dry weight, while the American cultivar surpasses the Iraqi cultivar in: number of branches, number of leaves, leaves area, shoot fresh weight, shoot dry weight and proline concentration.

KEYWORDS: Vigna unguiculata, Proline. Drought, Abiotic stress, Water stress.

المستخلص

أجريت الدراسة الحالية خلال موسم نمو واحد ابتداءً من اذار ولغاية تموز من العام ٢٠٢١ في احدى الحقول المحلية تحت الظروف الطبيعية باستخدام اصص بلاستيكية في قسم علوم الحياة، فاكولتي العلوم، جامعة زاخو، إقليم كوردستان العراق. ضمت التجربة دراسة تأثير نقع صنفين يزرعان محليا لنبات اللوبياء هما الصنف العراقي والامريكي في ٢٥٠ جزء بالمليون لحامض السالسيلك والماء المقطر كلا على حدة لمدة أربع ساعات لكل منها قبل الزراعة، فضلا عن استعمال الرش الورقي بنفس التركيز لحامض السالساليك في ثلاث مراحل: مرحلة النمو الخضري (الورقة الحقيقية الرابعة) وبداية مرحلة الازهار إضافة لمعاملة المقارنة والتي تمثلت بعدم الرش. اما العامل الاخر فقد تمثل في تعريض النبات الى مستوين من الجفاف هما ٣ و ٥ أيام من الجفاف في بداية مرحلة التزهير إضافة الى معاملة السيطرة التي تمثلت بالري المستمر يوميا للوصل الى ٧٥% من السعة الحقلية لغاية يوم الحصاد في نهاية التجربة. وأظهرت النتائج ان نقع البذور في حامض السالسيلك قد شجع وحفز كل من نسبة انبات البذور، ارتفاع النبات، عدد الازهار، الوزن الرطب للمجموع الخضري، الوزن الرطب للمجموع الجذري، الوزن الجاف للمجموع الخضري، الوزن الجاف للمجموع الجذري فضلا عن زيادة تركيز البرولين. في حين لوحظ اختزال النمو نتيجة لفترات الجفاف التي تعرض اليها النبات باستثناء اطوال الجذور التي ازدادت بزيادة مدة الجفاف. كما وجد ازدياد تركيز البرولين بزيادة فترة الجفاف من ٣ الى ٥ أيام بالمقارنة مع معاملة الري المستمر، ومن ناحية أخرى فقد حفز النقع والرش بحامض السالسيلك الى زيادة تركيز البرولين. وأوضحت النتائج أيضا تفوق الصف العراقي على الصنف الأمريكي في نسبة انبات البذور، ارتفاع النبات، عدد الازهار، الوزن الرطب للجذور وكذلك اطوالها والوزن الجاف للجذور، في حين تفوق الصنف الأمريكي في عدد الافرع الخضرية، عدد الأوراق، مساحة الأوراق، الوزن الرطب للمجموع الخضري والوزن الجاف للمجموع الخضري فضلا عن تركيز البرولين في الانسجة المعرضة للشد المائي.

INTRODUCTION

Cowpea grain nutrition is essential since it is consumed in large quantities by millions of individuals whose diets are deficient in protein, minerals, and vitamins. In comparison to cereal grains, cowpea grain protein is high in the amino acid lysine and tryptophan. The grain is very high in minerals and vitamins, and it has one of the highest quantities of folic acid of any diet, which helps prevent spinal tube abnormalities in unborn infants (Hall *et al.*, 2003). Cowpeas can be utilized as a vegetable crop at any stage of development, and the leaves have a high nutritional value (Ahenkora *et al.*, 1998; Nielson *et al.*, 1993). The soft green leaves are a staple diet and are used as a pot herb similar to spinach. Immature green pods are often combined with cooked dry cowpeas or other meals in the same manner that snap beans are. Cowpea foliage is a major source of high-quality hay for animal feed in many parts of the world (Tarawali *et al.*, 2002).

Drought is regarded as a major limitation to crop production in most developing countries and it occasionally causes agricultural losses in developed countries (Ceccarelli and Grando, 1996). Drought stress has been researched in numerous crops, drought stress also affects the availability and transport of soil nutrients, as water transports nutrients to the roots. However, drought in particular, causes a serious threat to sustainability of agricultural production.

Salicylic acid has a high metabolic and physiological activity, allowing it to play a regulatory role in plant development and response to biotic and abiotic stress stimuli. SA is found in plant tissues in amounts ranging from several mg to several ng per gram of fresh mass in non-stress conditions. Its concentration rises dramatically in plants that are deficient in water.

Proline has long been thought to be an innocuous, compatible osmolyte that protects subcellular structures and macromolecules from osmotic stress (Kavi and Kishor, 2005). Proline buildup, on the other hand, can affect stress tolerance in a variety of ways. Proline has been proven to operate as a molecular chaperone, protecting protein integrity while also enhancing enzyme activity. The current study aims to determine the effect of drought and salicylic on different vegetative growth and proline accumulation in addition to identify the more resistant cultivar to water deficiency.

MATERIALS AND METHODS

The present study was carried out during one growing season from March to July, 2021 under field condition in local farm under field conditions in Biology Department/ Faculty of Science / University of Zakho -Kurdistan Region of Iraq.

Two local culturing cultivars were used, local Iraqi and American one obtained from local agricultural markets. Plastic pots (20cm height, 18cm diameter) were used, each pot was filled with 4 kg of mixture of soil and manure (250 mg/ 4kg soil) as recommended by farmers.

Factorial Randomized Complete Block Design (RCBD) was used for statistical analysis, the current study includes examining effect of four factors. First, two cultivars of local cowpea seeds cultured annually by Kurdistan region farmers. Second was soaking the seeds into 250 ppm of salicylic acid and distal water for four hours before planting. Third represents utilizing foliar spraying of 250 ppm of salicylic acid at three stages, at vegetative (4^{th} true leaf stage), at the starting of flowering stage and the third was without spraying as a control treatment. The fourth factor was investigating the effect of drought which in three levels, control with continuous irrigation, three and five days of water withholding at flowering stage,

followed by continuous daily irrigation (75% field capacity) till the harvest date of the experiment.

Four uniform and healthy seeds of cowpea were cultured in each plastic pot with three replicates for each treatment in March, 8, 2021, which was thinned into two (2) seedlings per pot after two weeks of culturing date. The followed parameters were measured: Seed germination, branches number, number of leaves, leaf area (mm^2), length of plant, number of flowers, shoot fresh weight (gm), root fresh weight (gm), length of roots, shoot dry weight (gm), root dry weight (gm) and Proline content (µmoles. g) which was calculated according to Bates *et al.*, (1973). The data of the experiments submitted to SAS Software (SAS, 2016), the differences between means and studied characteristics were compared using Duncan multiple range test (Duncan, 1955).

Seed germination

Findings of Figure (1) illustrate that there are highly significant differences between the two cultivars, that Iraqi local cultivar exceeds the American which records 59.72% in the seeds immersed in water compared with the American which records 45.83%. On the other hand, another significant difference was found between the same cultivar type and immersion treatment interaction at for the Iraqi cultivar when they were immersion at water for 4 hours before planting which reached 59.72% compared with 52.77% with those seeds immersed in 250 ppm of salicylic acid solution. Therefore, it's clear that the water increases the germination percentage compared to salicylic acid. Meanwhile immersion the seeds of Iraqi cultivar in water beat the American seed immersed in both water and salicylic acid.

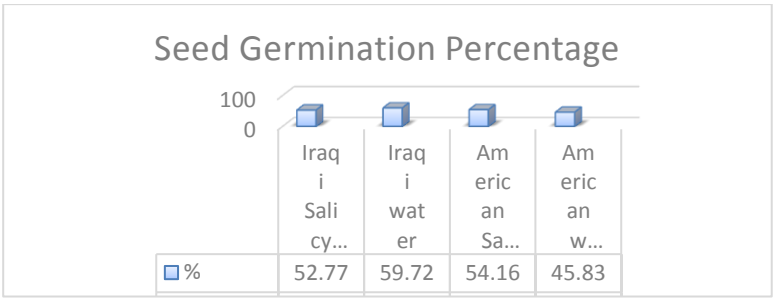

Figure (1): Seed germination as a result of salicylic and drought effects: (The significant difference for Cultivar means).

Branches number

Significant differences were found in the branches number as a result to studded factors and its interactions. From the data, the cultivar, immersion, and drought affected the branches number significantly (Figure 2). Regarding cultivar type, the American significantly surpass the Iraqi cultivar by recording 7.0 while the Iraqi gain 6.6 branches. Relating to the drought effects, there was generally significant difference between control and 5 in addition between 3 and 5 days of water shortage. As well, significant effects were found between the spraying in vegetative stage and control. Meanwhile, the immersion in water records more branches compared to salicylic acid significantly.

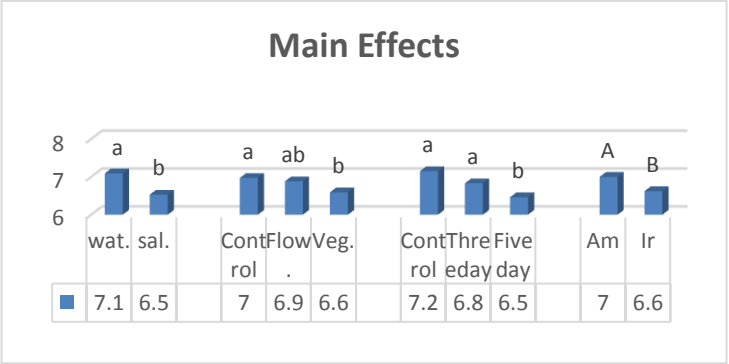

Figure (2): Branches number as a result of salicylic and drought effects: (The significant difference for cultivar, immersion, spraying and drought)

Leaves number

Two significant differences were found concerning the leaves number, Soaking in both salicylic acid and drought period. From the figure (3) it's clear that immersing the seed for 4 hours in water cause significant positive effects by increasing the numbers of leaves to 21.54 compares with those immersed in salicylic acid (19.65). Otherwise, water deficit for 5 days causes significant reduction in the number of leaves to 19.5 compared to control (21.6) with continuous irrigation. Furthermore, non-significant differences were seen between all the interactions of examined factors, in spite of the fact, the outcomes indicated that the leaves number were decreased when the drought period increased to 3 days and 5 days of dryness when compared with control treatment in general.

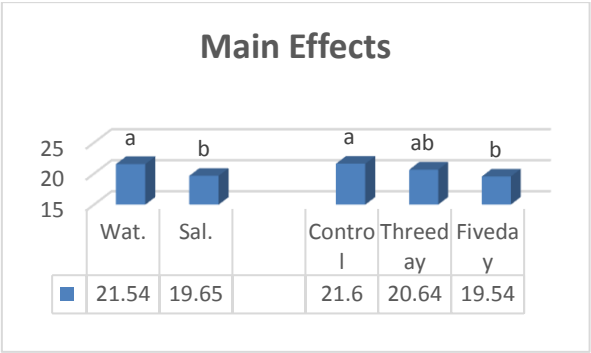

Figure (3): Leaves number as a result of salicylic and drought effects: (The significant difference for immersion and drought).

Leaves area

Highly significant differences (Figure 4) were found in leaves area as a result of different cultivars, immersion treatment, time of spraying and drought. The mean value of leaves area in American cultivar records around 50.52 mm^2 while the Iraqi cultivar reached 35.02 mm^2, at the same time, seeds immersion in water stimulates significantly increasing the leaves area to 49.37 mm2 when compared with immersing in salicylic acid (36.17mm^2). Furthermore, the plant without spraying gained large area (49.02mm^2) contrast to vegetative (36.14mm^2) and flowering stages (43.15mm^2) respectively. In addition, subjecting plants to desiccating for 5 days reduce the area significantly to 38.24mm^2.

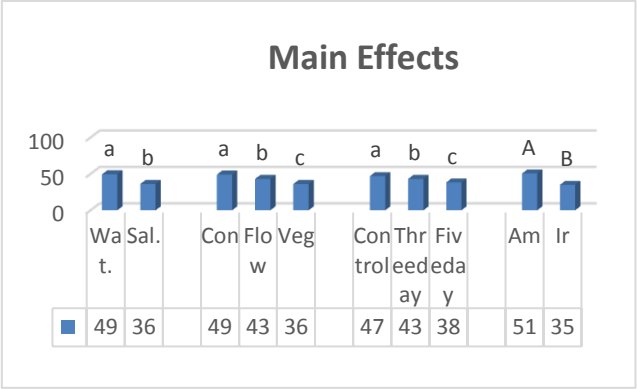

Figure (4): Leaves area (mm^2) as a result of salicylic and drought effects: (The main significant effect of Cultivar, Immersion, Spraying and Drought)

Plant length The data indicated that all studded factors and their interactions are highly significant except (Cultivar × Drought) one. Therefore, the Iraqi cultivar exceeds the American which they record 17.12 cm and 16.92 cm respectively (Figure 5). Furthermore, soaking the seeds in salicylic acid gain higher length (17.19 cm) compared with (16.23 cm) in plants with seeds immersed in water. On the other hand, significant difference was recognized according to spraying time and drought exposing period.

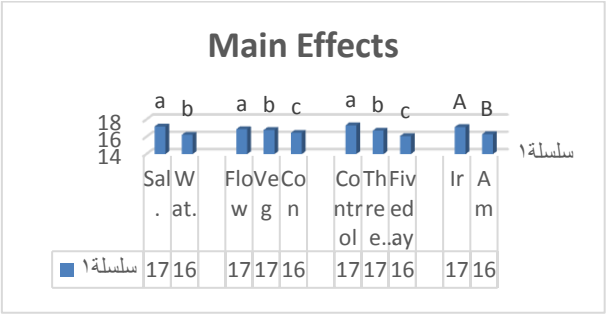

Figure (5): Plant length (cm) as a result of salicylic and drought effects: (The significant main effects cultivar, soaking, spraying and Drought)

Flowers number

Figure (6) illustrates that the immersion, spraying and drought appeared significant differences in the flowers number. It looks alike that soaking the seeds in salicylic acid records 2.7 flowers/ plant while immersing them in the water for the same period records 2.29 flowers. Meanwhile, spraying in vegetative show higher number 2.8 followed by flowering stage 2.5 and 2.2 at control treatment 2.12 respectively. Meantime, rising the drought period to 3 and 5 days reduce the number of flowers from 2.5 to 2.09 respectively.

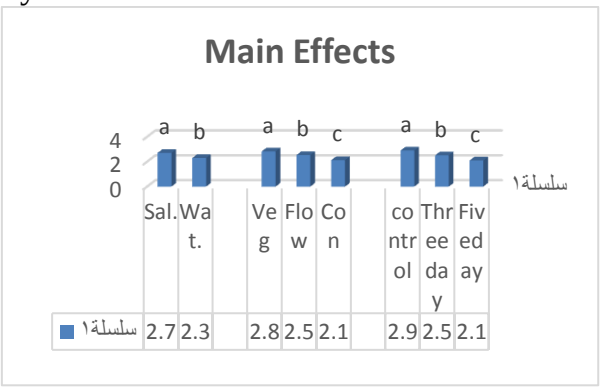

Figure (6): Flowers number as a result of salicylic and drought effects: (The significant main effects of Immersion, spraying date and drought)

Shoot fresh weight

Fresh weight of the shoots was affected significantly by soaking, spraying date and Drought but not affected as a result to cultivar type. Figure (7) detected that the seeds immersed with salicylic acid increase the fresh weight significantly by recording 2.77 gm / plant compared by 2.4 gm in the plants with seeds soaked in water for same period. Likewise, the spraying at flowering (2.69 gm) and vegetative (2.12 gm) stage reduces the fresh weight significantly parallel to non-sprayed plants. Additionally, valuable significant decrease was clear in the fresh

weight to 2.46, 2.05 gm contrast to 3.28gm in control treatment by increasing the water deficit for 3 to 5 days.

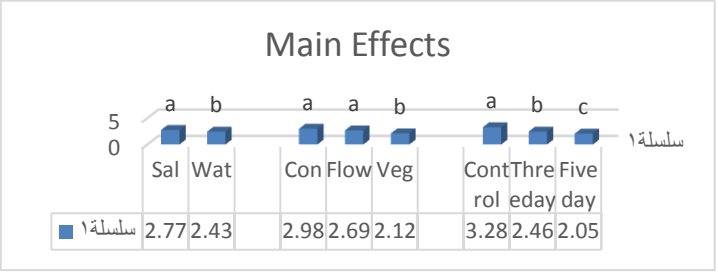

Figure (7): Shoot system fresh weight (gm) as a result of salicylic and drought effects: (The main significant effect of immersion, spray and drought)

Roots fresh weight

Fresh weight of the roots was affected significantly on cultivar type, Immersion, spraying date and Drought. Figure (8) clarify that the Iraqi cultivar roots grow better and significant than the American by recording 2.04 gm compared to 1.15 gm. Also, seeds immersed with salicylic acid increase the fresh weight significantly by recording 1.69 gm / plant compared by 1.5 gm in the plants with seeds soaked in water for same period. Additionally, non-significant differences were present between spraying at vegetative (1.67 gm) and flowering (1.64 gm) stages but were both more than the non-sprayed treatment (control, 1.47 gm). Furthermore, valuable significant decrease was clear in the fresh weight to 1.28, 1.59 gm contrast to 1.91gm in control treatment by increasing the water deficit for 3 to 5 days.

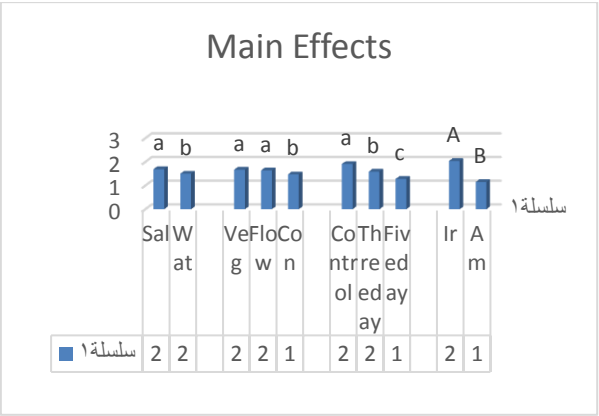

Figure (8): Root fresh weight (gm) as a result of salicylic and drought effects: (The main significant effect of Cultivar, immersion, spray and Drought)

Root Length

All the main factors and their interactions were obviously indicated that the roots length is highly affected. Staring on the figure (9) which indicate that the Iraqi cultivar exceeds significantly the American in the length of the roots which was reached 18.22 and 17.42 cm respectively. On the other hand, non-sprayed plants (18.1 cm) gain taller roots compared with those sprayed at vegetative (17.48 cm) and flowering (17.87 cm). At the same time, it was clear that treating seeds with salicylic acid will stimulate the plants for forming taller roots by recording 18.36 cm which differs significantly on those soaked in water only that reached 17.28 cm. Regarding the effects of increasing the water shortage period from 3 to 5 days, promote the plants to increase the length significantly which reached 17.77, 18.79 cm respectively in contrast to control treatment with continuous irrigation (16.89cm)

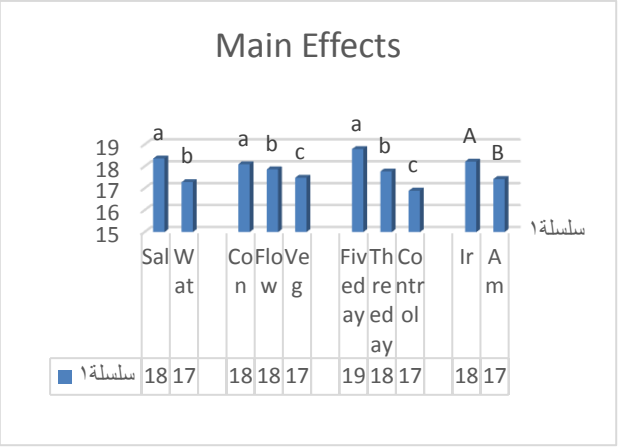

Figure (9): Root length as a result of salicylic and drought effects: (The significant main effects of Cultivar, Immersion, Spraying and Drought)

Shoot dry weight

Interesting results were documented in shoot dry weight, that all main studded factors and all its interactions were highly significant. From the Figure (10) which revealed that the American cultivar (1.03 gm) exceeded the Iraqi (0.83 gm) in dry weight. Meanwhile, it was clear that soaking the seeds in salicylic acid will stimulate significantly shoot proliferation which records 1gm / plant contrast to immersing in water (0.86). Moreover, 1.03, 0.81 and 0.95gm were recorded as a result of non-sprayed, sprayed at vegetative and flowering stage respectively. At the same time, a significant gradual decrease (0.89 and 0.75 gm were obvious due to increasing the water stopping for 3 and 5 days respectively parallel to control treatment (1.14 gm).

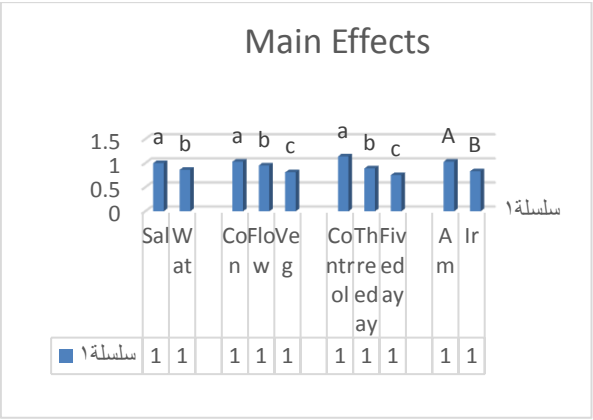

Figure (10): Shoot dry weight (gm) as a result of salicylic and drought effects: (The main significant effect of Cultivar, immersion, spraying and drought)

Root dry weight

Similar response was showed in the effects of studded factors of root dry weight to shoot dry weight that all main factors and all its interactions were highly significant. Based on the Figure (11) is revealed that the American cultivar (0.6 gm) records less amount than the Iraqi (0.75 gm) that exceeded in root dry weight. Meanwhile, it was clear that soaking the seeds in salicylic acid will promote significantly root mass production which records 0.73gm / plant contrast to immersing in water (0.63). Moreover, 0.72, 0.61 and 0.72gm were recorded as a result of non-sprayed, sprayed at vegetative and flowering stage respectively. Meanwhile, a significant gradual decrease (0.7 and 0.5 gm) and reduced due to increasing the water shortage for 3 and 5 days respectively compared to control treatment (0.84 gm).

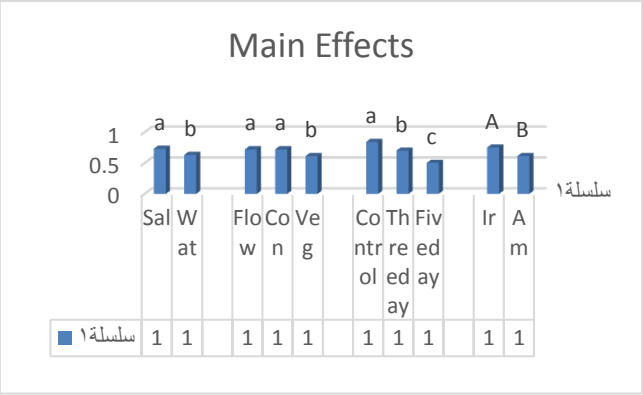

Figure (11): Root dry weight (gm) as a result of salicylic and drought effects: (The main significant effect of Cultivar, immersion, sprayed and Drought)

Proline Concentration (µmoles/ gram)

The results revealed that there were significant effects according to the cultivar and drought period in proline concentration in the cowpea leaves (Figure12). Therefore, the amount of proline in American leaves tissue accumulated in amount of 0.08 µmoles/ gram which differs significantly in the concentration inside the Iraqi cultivar leaves (0.05 µmoles/ gram). On the other hand, the concentration was increased significantly from 0.03 µmoles/ gram in control treatment to 0.06 µmoles/ gram when the plants subjected to 3 days of water scarcity and 0.11 µmoles/ gram when drought extended to 5 days of dryness.

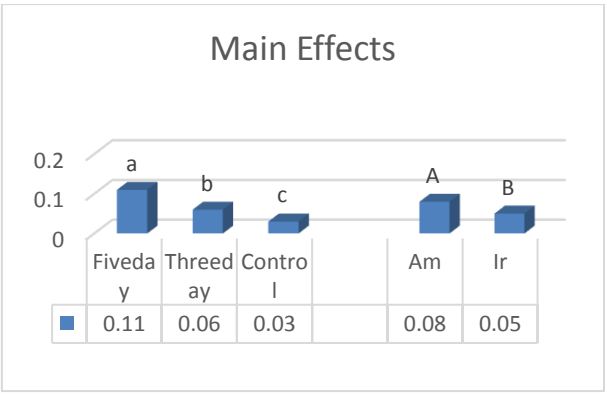

Figure (12): Proline concentration (µmoles/ gram) as a result of salicylic and drought effects: (The main significant effect of Cultivar and Drought)

DISCUSSION

Back to previous results regarding the effect of drought on cowpea growth parameters, the current study indicate that all growth traits decreased as a result to increase the drought stress by exposing the plants to gradual increase of water deficiency from 3 to 5 days during flowering stage compared with the continuous irrigation of 75% of field capacity. This reduction is due to decrease of water which cause impaired germination and poor stand establishment (Harris *et al.*, 2002). Reduced turgor pressure that causes cell proliferation, which is one of the most drought-sensitive physiological processes (Nonami, 1998). In addition to that, the water shortage will have negative impact on grain output when it coincides with the reproductive stage (Thomas, 1997). Moreover, the cell division, expansion, and enlargement aid growth while also making them vulnerable to drought stress. Meanwhile, water deficit will cause leaf senescence and abscission by reducing leaf formation and growth (Karamonos, 1980).

Evaluating the effects of soaking the cowpea seeds in 250 ppm of salicylic acid for 4 hours before planting, the outputs of

almost examined parameters clarify that each of: seed germination percentage, plant length, number of flowers, shoot fresh weight, fresh root weight, roots length, shoots dry weight and roots dry weight were stimulated and increase the amount of each of them. This increment and these activating effects of SA may due to of its favorable effect on plant germination and growth of shoots and, in particular, roots of plant species (Shakirova, 2007). In addition, it was mentioned that the exogenous SA will increases in photosynthetic rates following spray treatments due to enhanced enzyme activity linked to CO_2 uptake at the chloroplast level rather than simple stomatal opening (Khan *et al.*, 2003).

Concerning the significant increase of proline under stress conditions and referring to the previous results related to the superiority of the Iraqi variety in the growth characteristics of the root system and the accumulation of a greater amount of proline, as well as field observations, it can be considered the most drought-resistant variety than the American variety. Thus, the plant will develop a physiological mechanism to overcome and resist the stressed unfavorable conditions by increasing the concentration of accumulated proline which is a low molecular weight organic solutes known as suitable osmolytes needed for osmotic adjustment (Naidu *et al.*, 1992).

Concerning the differences between the two studded cowpea cultivars, the findings clarified that the Iraqi cultivar exceed the American cultivar in seed germination percentage, plant length, number of flowers, root fresh weight and root length. While the American cultivar surpasses the Iraqi cultivar in: number of branches, number of leaves, leaves area, shoot fresh weight, shoot dry weight and proline concentration. This difference between the two cultivars may be due to diverse genetic makeup of them, in addition to acclimatization and well adapted to the regional climate environment.

CONCLUSION

According to previous results it can conclude that immersing the seeds in suitable concentration of salicylic acid before planting will stimulate seed germination may promote many other traits while it may be considered as a high concentration which will inhibit some vegetative growth traits. Furthermore, subjecting the plants at flowering stage to for water deficiency will reduce growth parameters and will increase the roots length. Meanwhile, Proline concentrations increased by rising the period of water withholding and treating plants with salicylic acid will increase proline accumulation in stressed plant leaves. Finally, each plant species has his own genetic diversity which will adapted and grow in different behavior according to the regional climate and environment.

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