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Stimulating the growth of (*Acacia arabica* Lam.) seedlings growing under water stress conditions chemically using proline and salicylic acid

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ABSTRACT

(The experiment was carried out during the period from the cultivation of seeds on 21-2-2017 to 10-4-2018 where the seeds of Acacia arabica Lam. Seeds were planted inside the lath-house at the Faculty of Agriculture-University of Kerbala in Husseiniya district, Karbala, Iraq to examine the effect of proline acid (0, 75, and 150 mg.L^{-1}) and salicylic acid (0, 50 and 100 mg.L^{-1}) on this plant under water stress. The results showed that spraying the seedlings with proline at concentration of 150 mg.L^{-1} increased the relative moisture content and the total chlorophyll content in leaves with an increase ratio of (7.15 and 30.76%) respectively compared to the control treatment. The treatment of seedling with salicylic at concentration of 100 mg.L^{-1} of had a significant impact on all studied traits compared to the control treatment. The results also revealed the superiority of the interaction treatment between proline at a concentration of 150 mg.L^{-1} and salicylic at concentration of 100 mg.L^{-1} in all studied traits relatively to the control treatment. Foliar spraying of organic acids could be a relief treatment to maintain the growth and development of plants under environmental stresses particularly at the early stages of growing and flowering)

Key words : *Acacia arabica, Salicylic acid, Water stress proline*

Introduction

Acacia species is floral and dicotyledonous plant belong to the legume class. The Acacia genus contains about 1300 plant species of which 960 are native to Australia and the rest are distributed around temperate-warm regions in the northern and southern hemispheres (Farzana *et al.,* 2014).

The Arabic acacia tree (*Acacia arabica* Lam.)is medium-sized trees and native to Africa and Asia with dark stems, long white thorns, and evergreen leaves. Its Yellow flowers appear in the spring and summer, and will be ended with horny fruits. It is often planted in shade on the sides of agricultural roads, sides of streams and rivers. Ithas the ability to tolerate drought to some extent and grow in sandy, salty and stony land. It is propagated with seeds (Rajvaidhya *et al.*, 2012).

Water stress is one of the most important types of environmental stresses that occur when soil water is low due to lack of rain or when the loss of water by transpirationis superior to the absorption of water by the roots, so drought is a major abiotic factors that affect the reduction of plant productivity around the world and causes significant agricultural losses especially in arid and semi-arid areas

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(Al-Ghanmi *et al.,* 2015).

It is well known that the water stress is three levels, the first is mild and the second is moderate stress, and the third is severe stress, which negatively affects the indicators of vegetative growth by stimulating the formation of free radicals with oxidative effect on the plantcells and thus turns to multiple stress, which includes waterstress, as well as Oxidation Stress, which exacerbates the negative effects on growth indicators (AL-Taey and Saadoon, 2014; Al-Saadi and Almuntafji, 2016).

Plants exposed to environmental stress often exhibit significant physiological and biochemical changes that negatively affect plant growth and the functions of its various organs. Water shortages are known to be one of these stresses, which are often the result of drought conditions in the plant growth environment, which cause morphological and physiological problems for the plant life cycle and productivity (AL-Taey *et al*., 2010). The ability to adapt and respond to stress is accompanied by the phenomenon of osmosis regulation, which depends on the accumulation of organic and mineral compounds at the cellular level, which allows the water balance of the plant to tolerate the stresses expected to be exposed to that plant within the environment of growth (Yassin, 2001; Galme´s *et al*., 2007; AL-Taey, 2017).

Proline acid is one of the natural essential amino acids in the composition of proteins and is a non-polar amino acid different from its counterparts in the rest of the other amino acids. Proline is distinguished from 20 amino acid in a unique synthetic formula in which the amino group NH_2 is not free and this means that it has a secondary function, not a primary one. Several studies have shown that proline acid is one of the most important amino acids in the plant. The most important characteristic of this acid from the other amino acids is its containment of a secondary amino acid group. This group is free in all other amino acids except for proline (Al-Dalali, 1980) and is significantly accumulated when the plant is exposed to many environmental stresses compared to other amino acids (Mattioli, 2009; Burhan andAL-Taey, 2018).

Salicylic acid is a phenolic natural compound and a part of the pathways induced in the plant by many of the plant's biological stresses (AL-Taey, 2009). It is involved in a wide range of metabolic activities in plants. It has recently been known that salicylic acid produces phenolwhich acts as a barrier in the plant cell wall against loss of moisture (Burguieres *et al*., 2007).

This acid is classified under the group of internal plant hormones because it has an important physiological role in plant growth, flowering, ion absorption, activation of chlorophyll and carotene pigments, and accelerating the process of photosynthesis (Hayat, 2007).

As Iraq has been and is still a country that spends large amounts in the establishment of irrigation networks to control water scarcity and the worsening problem of water stress in recent years due to drought and lack of rainfall and the importance of these plants,the study aims to study the effect of proline and salicylic acid in improving the qualities of growth under the influence of water stress.

Materials and Methods

The experimentwas conducted inside the lath-house at the Faculty of Agriculture- University of Kerbala in Husseiniya district, about 14 km north of the holy city of Karbala, Iraq (longitude 58>0644° east and 17>3232°north and at 29 m above sea level) during the period from seed planting on 21/2/2017 to 10/4/2018. Certified local seedsof *Acacia arabica*Lam. With high germination percentage were planted using plastic bags with a volume of 2 kg.

The plants were treated withprolineat three concentrations (0, 75, and 150 mg.L^{-1}) andsalicylic acid at three concentrations (0, 50 and 100 mg.L^{-1}). The trial wasa factorial experiment(3×3) and designed according to the Randomized Complete Block Design with three blocks. The number of experimental units was 27 and each experimental unit hadfive observations thus the total number of observations in each experiment was 135 observations.

Seedlings of both species were exposed to water stress in September 2017 when they were about 7 months old. The field capacity was estimated in the soil used in the study by selecting three bags packed with 2000g soil/bag. The bags were completely drained under the sunthen the soil was watered until full saturation and left for 48 hours.The amount of water vapor was reduced by placing a black plastic bag as a cover on each bag of the three polythene bags. The bags were left until the last drop of sedimentary water was removed through the bottom holes of the bags and then weighed again.

The level of 50% of the field capacity was used in the gravimetric method according to the method in Klute (1968) and (Al-Ghanami *et al*., 2015) and calculated as follows:

Lost water weight= wet soil weight- dry soil weight

The percentage of water in 2000 g soil/bag =× 100

$$= \frac{\text{The weight of the lostwater}}{\text{Dry soil weight}}$$

Water lost in the first bag: 2450 - 2000 = 450
Water lost in the second bag: 2432 - 2000 = 432
Water lost in the third bag: 2375 - 2000 = 375
Where the field capacity in the first bag was 22.5
Where the field capacity in the second bag was 21.6
Where the field capacity in the third bag was 18.75
The field capacity of the soil is 20.95%. As 50% of the field capacity is required. The average amount of water added to each sack of sacks is 419 mL/2 = 209 mL.

The studied properties

Determination of total ash content in leaves (%)

The ash content was determined by the procedure by using AOAC (1995). Twograms of powdered leaves were taken from the studied species and placed in a ceramic vase and burned in the oven at a temperature of 600 º C until the sample color turned to the slanted gray to the white and then left until cold and then weighed. The percentage of ash in the leaves was estimated based on dry weight according to the following equation:

$$\% \text{ Ash} = \frac{\text{Weight of ash}}{\text{Dry sample weight}} \times 100$$

2. Relative Water Content (RWC) (%)

The moisture content of the leaves was estimated as described by (Gonzalez and Vilar, 2001) by taking weight of 20 fresh and wet leaves for each seedling in the experimental unit and the wet weight was recorded directly. The leaves were then immersed in distilled water for 24 hours at room temperature and under low light conditions to saturate the leaves with distilled water and their bloating weight was recorded in saturationcondition. After that, the leaves were dried in the oven at 70 °C until weight stability and dry weight was recorded. The relative moisture content of each treatment was calculated according to the following equation:

$$\textbf{RWC \%} = \frac{\textbf{Fresh Weight} - \textbf{Dry Weight}}{\textbf{Turgid Weight} - \textbf{Dry Weight}} \times \textbf{100}$$

Determination of leaf content of total chlorophyll (Mg/g^{-1}wet weight)

The total content of chlorophyll was estimated in fresh leaves based on McKinney (1941) and according to Shareef (2018) by Spectrophotometer at 645 and 663 nm. Using the equations below, total chlorophyll concentration was estimated in plant leaves and calculated on the basis of Mg.g^{-1}fresh plant tissue:

Total Chlorophyll = [20.2 (D645) £« 8.02 (D663)] × V/ 1000 ×W

Determination of leaf content of total proteins (%)

The percentage of proteins was estimated by estimating the percentage of nitrogen in the dry leaves using the Kjeldahl method, which consists of three stages according to Shareef (2018). Firstly, the stage of digestion, which is used according to Schuffelen *et al*. (1961) to convert nitrogenous materials to ammonium sulphate salt

Secondly, distillation phaseis used to react ammonium sulfate with sodium hydroxide and to release ammonia

The third stage is the titration to titrate the contents of the baker containing the distilled ammonia against HCL its standard 0.014 and calculate the added amount of HCL when the green mixture guide is changed to violet red .

The nitrogen ratio was then calculated by applying the following equation:

$$\text{N\%} = \frac{\text{Volume of HCL x Normality ofHCL x digested and diluted sample size} \times 14 \times 100}{\text{The size of the used extract x dry plant sample weight} \times 1000}$$

The percentage of nitrogen was then converted to the protein percentage based on the protein content of the nitrogen (conversion factor = 6.25) (AOAC, 1995).

% Protein = % N X 6.25

Statistical analysis

The data of this study were tested statistically using the Statistical Analysis Program SAS (2012)according to the design of the experiment. The data were subjected to Duncan's Multiple Range Test to compare the differences among the means whereas different letters indicate significant differences.

Results and Discussion

It can be seen from Table 1 that the concentrations of proline showed no significant differences between them in the ratio of total ashcontent in leaves. The same table showed that spraying seedlings with salicylicconcentrations significantly affected this characteristic whereas the concentrations of 50 and 100 mg.L^{-1} gave the highest percentage of total ash was 0.079 and 0.087%, respectively, compared to the control treatment which gave the lowest percentage of 0.069%. The data indicated that there wasa significant interaction between proline and salicylicin this characteristic, where it was found that the interaction between the concentration of 150 mg.L^{-1} of proline and 100 mg.L^{-1} of salicylic gave the highest rate of 0.091% compared to the control treatment, which recorded the lowest rate of 0.061%.

Table 2 shows that the concentrations of prolinehad a significant differences between them in the relative moisture content of leaves. The treatment of the spray with prolineat concentration of 150 mg.L^{-1} gave seedlings their leaves characterized by the relative moisture content with an average of 81.1% compared to the control treatment, which produced seedlings containing leaves with less moisture content at an average of 75.3%. All concentrations of salicylic showed differences that reached tosignificant level compared to the comparison treatment which produced seedlings with an average content of relative moisture content in their leaves 68.4% while the concentrations of 50 and 100 mg.L^{-1} of salicylic recorded rates of 79.2 and 86.0%, respectively. The data showed significant correlation between proline and salicylicin this trait, where the interaction between the concentration of 150 mg.L^{-1} of proline and 100 mg.L^{-1} of salicylic gave the highest rate of 87.6% compared to the control treatment, which recorded seedlings with the lowest moisture content of leaves at a rate of 60.0%.

The results of Table 3 indicate the significant differences in total chlorophyll content in fresh leaves

Table 1. Effect of proline and salicylic acids and their interaction on the total ash content (%) in leaves

Proline (mg/L^{-1})	Proline and Salicylic (mg.L^{-1})			Average of Proline
	Control	50	100	
Control	*0.061 c	0.074 abc	0.083 ab	0.082 a
75	0.076 abc	0.084 ab	0.087 ab	0.081 a
150	0.070 bc	0.081 ab	0.091 a	0.073 a
Average of Salicylic	0.069 b	0.079 a	0.087 a	

*The averages with the same letter for each factor and their interaction is a non-significant according to Duncan s multiple test at rang 0.05.

Table 2. Effect of proline and salicylic acids and their interaction on the relative humidity content (%) inleaves

Proline (mg.L^{-1})	Prolineand Salicylic (mg.L^{-1})			Average of Proline
	Control	50	100	
Control	60.0 d	78.6 abc	87.3 a	75.3 b
75	70.6 c	78.0 abc	83.0 ab	77.2 ab
150	74.6 bc	81.0 ab	87.6 a	81.1 a
Average of Salicylic	68.4 c	79.2 b	86.0 a	

Table 3. Effect of proline and salicylic acids and their interaction on the total chlorophyll content (mg.g^{-1} wet weight) inleaves

Proline (mg.L^{-1})	Proline and salicylic (mg.L^{-1})			Average of Proline
	Control	50	100	
Control	0.86 c	1.01 bc	1.11 bc	0.99 b
75	1.04 bc	1.09 bc	1.09 bc	1.07 b
150	1.16 b	1.48 a	1.64 a	1.43 a
Average of Salicylic	1.02 b	1.19 a	1.28 a	

of seedlings when treated with prolineat 150 mg.L^{-1}, which gave the highest rate of 1.43 mg. g^{-1} compared with the control treatment, whose leaves had the lowest total chlorophyll rate of 0.99 mg.g^{-1}.All concentrations of salicyliccaused the production of seedlings rich in total chlorophyll pigment ata significant rate of 1.19 and 1.28 mg.g^{-1}, respectively, compared to the control treatment, which recorded the lowest rate of 1.02 mg.g^{-1}. It is noticed from the data of the interaction between the studied factors that the total chlorophyllcontentof fresh leaves increased when treated with prolineat 150 mg.L^{-1} with salicylic at 100 mg.L^{-1}, and also when treated with prolineat 150 mg.L^{-1} with salicylic at 50 mg.L^{-1} at a rate of 1.64 and 1.48 mg.g^{-1} respectivelycompared with the control treatment, which its seedlings recorded the lowest chlorophyll content of 0.86 mg.g^{-1}.

The results of Table 4 showed no significant difference in the content of total protein when treated with proline, although the highest percentage of protein was recorded at concentration of 150 mg.L^{-1}, which gave a rate of 18.196% compared to the control treatment, which gave seedlings whose leaves have a lower rate of proteins which was 16.820%. Seedlings with protein-rich leaves were under the influence of salicylicat concentration of 100 mg.$L^{-1}$with a significant rate of 20.217% compared to the control treatment, which recorded the lowest rate of 15.173%, with an increase percentage of 24.94%. Total protein content of leaves increased by 30.37% when 150 mg.L^{-1} of proline overlaps with 100 mg.L^{-1} of salicylic where this treatment gave a rate of 21.583% compared to the control treatment, which recorded low-protein seedlings amounted to 15.027%.

From the review of the data in Tables 2, 4 it can be noted that the treatment of seedlings with proline had nosignificant increase in the content of the leaves of ash and the total protein. This may be due to the accumulation of proline naturally in plants exposed to osmosisstress by stimulating its synthesis. This method is one of the most important means of using organic compounds by plants exposed to environmental stresses, especially drought stress (Delauney and Verma, 1993). Toorchi *et al.* (2011) has shown that amino acid proline accumulates in plants when exposed to water or saline stress and plays an effective osmosis protective role.

Salicylic acid had a major role in most studied traits. The reason is that salicylic is involved in the regulation of physiological processes in plants such as the closure of stomata, absorption and transfer of ions, transpiration, photosynthesis and the biogrowth of green plastids (Shakirova *et al.*, 2003). Salicylicalso has an effective and protective role in protecting green plastid membranes from free radicals and heavy metal toxins by activating antioxidants (Ananieva, 2004). Ali and Jaafer (2003) reported that the effect of salicylic on plant growth is due to its effect on the increase of enzymatic activity, especially catalase enzyme in leaves, which helps to increase the effectiveness of photosynthesis and increase the processed food in leaves. The effect of salicylic in increasing plant growth may be due to its active role in the biological regulation of element uptake, cell division and elongation, enzymatic activity, protein synthesis, and enhanced photosynthesis (Blokhina *et al*., 2003).

It is clear from the data of all the tables and both that there is a significant effect of the binary interference factors between the proline and salicylicin all studied traits. This may be due to the nature of the organic acids that work in a combination with other organic compounds such as proteins, pyrenes, pyrimines, alkaloids, vitamins and enzymes. The organic acid spraying on plants plays a major role in stimulating physiological and biochemical processes. The organic acids are involved in the building of proteins and the manufacture of carbohydrates by building chlorophyll and stimulating the process of photosynthesis. It also increases the plant resistance to thermal and water stresses and participates in building and encouraging the work of

Table 4. Effect of proline and salicylicacids and their interaction on the total protein content (%) inleaves

Proline (mg.L^{-1})	Prolineand Salicylic (mg. L^{-1})			Average of Proline
	Control	50	100	
Control	15.027 b	15.520ab	19.913 ab	16.820 a
75	14.883 b	18.393 ab	19.153 ab	17.477 a
150	15.610 ab	17.393 ab	21.583 a	18.196 a
Averageof Salicylic	15.173 b	17.102 ab	20.217 a	

many enzymatic enzymes (Shafeek Akron, 2012).

Conclusion

It can be concluded that this study approved the vital role of organic acids to overcome environmental stresses. Although some organic acids such as proline can be accumulated naturally during these stresses, spraying these acids could be an assistance to maintain optimal growth of plants exposed to abiotic stress. This study also revealed the importance of combination among organic acids in mitigating drought effect.

References

Al-Dalali, Basil K. 1980. Fundamentals of Organic Chemistry. Ministryof Higher Education and Scientific Research, University of Mosul.

Al-Ghanmi, Abd Aoun H.A., Abdul Jassim M. J Al-Jubouri and Qyood T. Y. Al-Asadi 2015. Reducing the effect of water stress using proline in terms of growth of some cultivars of corn. *Journal of Karbala University*. 13(2) : ....

Ali, G. and Jaafar, H. Z. E. 2013. Interactive Effect of Salicylic Acid on some physiological Features and Antioxidant Enzymes Activity in Ginger (*Zingiber officinale* Roscoe). *Molecules.* 18 (5): 5965 – 5979.

Al-Saadi, Abbas J. H. and Haidar N. H. Al-Montafji, 2016. The relationship between water stress, selenium and brassinolide in some indicators of vegetative growth and the content of elements in *Coriandrum sativum* L., *Ibn al-Haytham Journal of Pure and Applied Sciences*. 29 (2) :

AL-Taey, D. K. A. and Saadoon, A.H. 2014. Effect of treatment of Salicylic acid and water Salinity on the Growth and Nitrate Accumulation with nitrate reductase activity in the Leaves of Spinach, *Spenaciaoleracea* L. *Journal of Babylon University, Pure and Applied Sciences*. 3(22) : 1188-1203.

Al-Taey, D. K. A., AlAzawi, S. S. M. and Husien, M. H. 2010. Effect of Spraying Acetyl Salicylic Acid on the Plant Tolerance for Salt Stress & Survival Percentage after Transplanting of Orange (*Citrus sinensis*). *Babylon Journal University -Pure and Applied Science.* 18 (4): 1513-1520.

Al-Taey, D. K. A., S. S. M. 2010. Effect of spraying acetyl salicylic acid to reduce the damaging effects of salt water stress on orange plants (*Citrus sinensis* L.). *Journal of Kerbala University*. 7 (2) : 192-202

Al-Taey, D.K.A. 2017. Mitigation of Salt Stress by Organic Matter and GA3 on Growth and Peroxidase Activity in Pepper (*Capsicum annum* L.). *Advances in Natural and Applied Sciences*. 11 (10) : 1-11

Ananieva, E. A., Christov, K.N. and Popova, L.P. 2004. Exogenous Treatment with salicylic acid leads to increased antioxidant capacity in leaves ofbarley plant exposed to paraquat. *Journal Plant Physiol*. 161: 319–328.

AOAC. 1995. *Offificial Methods of Analysis* (16th ed.). Arlington, VA, USA: Associationof Offificial Analytical Chemists

Blokhina, O., Virolainen, E. and Fagerstedt, K.V. 2003. Antioxidants, oxidative damage and oxygen deprivations stress. *Ann. Bot.* 91: 179-194.

Burguieres, E., McCu, P., Kwon, Y.I. and Shetty, K. 2007. Effect of vitamin C and folic acid on seed vigour respondent phenolic – linked antioxidant activity. *Bioresource Technology.* 98 (7) : 1393 – 1404.

Burhan A. K. and AL-Taey, D. K. A. 2018. Effect of Potassium humate, humic acid, and compost of rice wastes in the growth and yield of two cultivars of Dill under salt stress conditions. *Advances In Natural And Applied Sciences*. 12 (11) : 1-6

Delauney, AJ. and Verma, D.P.S. 1993. Proline biosynthesis and osmoregulation in plants. *Plants Journal*. 4 : 215 – 223.

Farzana, M.U.Z.N., Tharique, I.A. and Sultana, A. 2014. A review of ethnomedicine, phytochemical and pharmacological activities of *Acacia nilotica* (Lim) willd. *J Pharmacogn Phytochem.* 3 : 84-90.

Galmes, J., Pou, A., Alsina, M.M., Tomas, M., Medrano, H. and Flexas, J. 2007. Aquaporin expression in response to different water stress intensities and recovery in Richter-110 (*Vitis* sp.): Relationship with ecophysiological status. *Planta.* 226 : 671–681.

Gonzalez, L. and Vilar, M. 2001. Determination ofrelative water content. *Handbook of Plant Eco-Physiology Techniques*. Netherland : Springer, 2001, pp. 207–212. ISBN 978-0-306-48057-7

Hayat, S., Ali, B. and Ahmed, A. 2007. Salicylic acid: Biosynthesis, Metabolism and physiological Role in plant. In: *Salicylicacid: A plant Hormone*. Springer, Netherlands. pp. 1–14.

Klute, A. 1986. *Method of Soil Analysis,* Part (1) and Monograph No. (9).

Mackinney, G. 1941. Absorption of light by chlorophyll solution. *Journal of Biological Chemistry*. 140 : 315-322.

Mattioli, R. 2009. The Proline biosynthetic genes P5C52 Play overlapping roles in Arabidopsis flower transition but not in embryo development. *Physio. Plant.* 137 : 72-85.

Rajvaidhya, S., Nagori, B.P., Singh, G.K., Dubey, B.K., Desai, P. and Jain, S. 2012. A review on Acacia arabica - An Indian medicinal plant. *Int. J. Pharm. Sci. Res.* 3 (7) : 1995 2005.

SAS. 2012. Statistical Analysis System, User' Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

Schuffelen, A. C., A. Euller and Vanschouenburg, J. G. 1961. Quick tests for soil and plant analysis used by small laboratories. *Netherland J. Agric. Sci.* 9 : 2-16.

Shafeek, M. R., Helmy, Y.I., Magda, A.F.S. and Nadia, M. O. 2012. Response of onion plants to foliar application of sources and levels of some amino acid under sandy soil condition. *Journal Appl. Sci. Rci.* 8 (11): 5521–5527.

Shakirova, F.M., Sakhabutdinova, M. V., Bezrukova, R. A. F. and Fatkhutdinova, D.R. 2003. Changes in the hormonal status of wheat seedlings induce by salicylic acid and salinity. *Plant Sci*. 164 : 317–322.

Shareef, S., Gazi, M. O. Abdullah and Zuhair A. Dawood, 2018. Effectiveness of treating of *Pinushalepensis* Mill. Seeds with electric shockand periods and Gibberellic Acid in changes of metabolic some the Physiological Processes of Seedlings. *Mesopotamia Journal of Agriculture.*

Toorchi, M., Naderi, R., Kanbar, A. and Shakiba, M.R. 2011. Response of spring canola cultivars to sodium chloride stress. *Ann. Biol. Res*. 2. 312–322.

Yassin, BassamTaha, 2001. Fundamentals of Plant Physiology. *Qatar National Library.* 46 (1) : 123-134.