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# Changes Induced in Some Physiological, Biochemical and Anatomical Characteristics of *Jatropha curcas* L. Plant by Exogenous Application of Osmo-Regulators Under Saline Irrigation Conditions.

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**ABSTRACT**

In response to rising oil prices and the quest for alternatives, economically viable and environmentally sustainable forms of energy, certain plants species with bio-energy potential had been proposed for large-scale planting and bio-fuel production. Hence, the present work was conducted in the greenhouse of National Research Centre, Egypt. During the two summer seasons of 2015 and 2016 to investigate the effects of three salinity levels (fresh water (0.23 $dSm^{-1}$), 3.13 and 6.25$dSm^{-1}$), and four osmo-regulators treatments (control (without osmo-regulators treatments),1000mg/liter Glutamine, 1000mg/liter Glycine betaine,500mg/L. Glutamine + 500mg/L. Glycinebetaine).Which sprayed two times during plant's life (the first after two months from planting and the second one-month later) and their interactions on growth parameters, leaf anatomy, total soluble solids percentage, osmotic pressure (Atm), photosynthetic pigments and total soluble sugars of *Jatropha curcas* L. Results illustrated that increasing salinity stress significantly reduced growth parameters of *Jatropha curcas* L. On the contrary, increasing salinity stress caused significant increases in most of leaf anatomy features, TSS, osmotic pressure, total soluble sugars percentage, and photosynthetic pigments content. Osmo-regulators treatments showed significant increases in all growth parameters, most of leaf anatomy features, photosynthetic pigments content and total soluble sugars percentage of *Jatropha curcas* L. leaf. While, TSS, and osmotic pressure revealed significant reduction in their contents with different osmo-regulators treatments as compared with control plants.

**Keywords:** Salinity, Osmo-regulators, *Jatropha curcas* L., Growth parameters, TSS, Photosynthetic pigments, Total soluble sugars, Leaf anatomy.

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## INTRODUCTION

Several environmental factors drastically affect plant growth development and yield performance of most crops(Bashan, 1998). Salinity was one of the most serious environmental problems reducing crop productivity (Patel *et al.* 2010). There were evidences that cells, tissues as well as organs, at different developmental stages of plant's growth exhibited varying degrees of tolerance to salinity stress(Munns 1993). Salt stress led to unfavorable functional changes and damage in plant tissues. Among other symptoms, changes in properties of membranes and disorganization in ions and metabolite transport were observed. Many metabolic pathways, such as photosynthesis and glycolysis were subject to modifications (Tozlu *et al.* 2000). In addition, high salinity reduced osmotic potential of the soil water and the availability of the soil water to the plant (Patel *et al.* 2010).Specific damage was associated with the accumulation of Na ions in leaves, which caused reductions in growth and yield of most crops because of the shortening of leaves lifetime; that in return reduced photosynthetic area, hence the photosynthesis, also fresh and dry weights of the plant (Munns, 2002). High salinity also caused osmotic damage because of the buildup of high concentrations of Na ions in plant leaves. Since Na ions entered leaves in xylem stream and was left behind as water evaporated (Garacia *et al*. 2010). Moreover, Salinity reduced growth through its bad effect on some physiological and biochemical processes, such as antioxidant phenomena, photosynthesis, ion homeostasis, nitrogen metabolism, and osmolyte accumulation (Ashraf, 2004 and Khalil and abou Leila, 2016).

*Jatropha curcas* L. (Euphorbiaceae) is a small tree and its probable center of origin is Mexico and Central America. International interest in *Jatropha curcas* L. as a fast growing, drought-tolerant, and renewable bio-energy crop has grown significantly in recent years (Gush 2008)**.** It soil is an environmentally safe and cost-effective renewable source of non-conventional energy and promising substitute for diesel, kerosene and other fuels. International interest in *Jatropha curcas* L. has grown due to a number of factors, including increased petroleum price, and reducing CO2 emissions and fuel security. This plant is also a source of poisons and medicines (Mandpe *et al.* 2005).

Amino acids or osmo- regulators were considered as precursors and constituents of proteins (Rai, 2002), which were important for stimulation of cell development. They acted as buffers, which maintain favorable pH within the cell (Sadak *et al*., 2015). In addition, amino acids were a well-known bio stimulant, which important for plant growth and yield also they mitigated the injuries resulted from stresses (Kowalczyk and Zielony, 2008 and Sadak *et al*., 2015).Glutamine was important as a constituent of proteins and as a central metabolite for amino acid transamination via α-keto glutarate and glutamate. Glutamine played an important role in the carbon skeleton and nitrogen exchange among different plant tissues, where this amino acid fulfilled many different physiological functions within the plant. When energy demands were high and glucose levels were low, cells could metabolize amino acids for energy. In addition, glutamine was one of the major sources of energy for many rapidly dividing cell types and it was most readily available amino acids for use as an energy source (Kazemi, 2013). Glycine betaine was the most popular compatible solutes that contributed to osmotic adjustment, and stabilization and protection of proteins, enzymes and membranes from the damaging effects of salt stress (Ashraf and Foolad, 2007). Glycine betaine was one of several such compatible solutes that had an osmo-protective function and was known to improve salt stress tolerance in most crop plants (Hossain and Fujita, 2010).Amino acids helped to increase chlorophyll concentration leading to higher degree of photosynthesis, which made cropslush. Amino Acids acted as a cytoplasm osmotic agent of stomata's cell, which helped plants to improve absorption of macro and trace nutrients as well as gasses through favoring the opening of stomata (Kandil *et al*., 2016).The aim of this work was to improve the salt tolerance of *J. curcas* plant that may allow it to grow and survive under saline irrigation conditions by exogenous application of glutamine and /or glycine as osmo-protectant.

## MATERIALS AND METHODS

### Experimental procedures

This study was conducted at the wire-house of the National Research Centre, Dokki, Cairo, Egypt, during two successive summer seasons; 2015 and 2016. *Jatropha curcas* L. Seeds were obtained from the Agricultural Research Centre, Ministry of Agriculture and Land Reclamation, Egypt. *J. curcas* seeds were selected for uniformity by choosing those of equal size and with the same color. The selected seeds were washed with distilled water, sterilized with 1% sodium hypochlorite solution for about 2 min and thoroughly

washed again with distilled water. One air-dried *J. curcas* seeds was sown at the center of each pot at 30-mm depth in earthenware pots, each filled with about 10 kg clay soil mixed with sandy soil in order to reduce compaction and improve drainage. Saline water was prepared by using sea salt with fresh water (0.23 $dSm^{-1}$) to achieve salinity levels of 3.13 and 6.25 $dSm^{-1}$. Concentration of EC, pH, cations and anions of soil used in the pot experiment are shown in Table 1.Granular ammonium sulphate 20.5 % N at a rate of 40 kg N ha-1, and single superphosphate (15% P2O5) at a rate of 60 kg P2O5 $ha^{-1}$ were added to each pot. The N and P fertilizers were mixed thoroughly into the soil of each pot immediately before sowing.

**Table 1: Mechanical and chemical analyses of the soil used during the experiment**

| | | |
|---|---|---|
| sand% | 25 | 25.7 |
| silt% | 35 | 35.3 |
| clay% | 40 | 39 |
| texture | clay loam | clay loam |
| F.C.% | 30.7 | 31.2 |
| W.P.% | 16.5 | 14.3 |
| CaCo3 | 4.4 | 4.3 |
| OM% | 1.4 | 1.1 |
| PH | 7.4 | 7.7 |
| EC | 0.7 | 0.6 |
| $Na^{+}$ | 2.2 | 2.1 |
| $Mg^{++}$ | 0.88 | 0.85 |
| $Ca^{++}$ | 1.11 | 1 |
| $K^{+}$ | 1.8 | 1.52 |
| $HCO^{-3}$ | 1.2 | 1.04 |
| $Cl^{-}$ | 0.57 | 0.65 |
| $CO3^{--}$ | 2.34 | 2.21 |
| $SO^{-4}$ | 1.85 | 1.55 |

**Treatments:**

**Water Treatments:**

The following three water treatments were applied throughout the entire growth period of the crop:

S0= Fresh water (0.23 $dSm^{-1}$)

S1=3.13 $dSm^{-1}$

S2=6.25 $dSm^{-1}$

Soil field capacity in the pots was estimated by saturating the soil in the pots with water and weighing them after they had drained for 48 h. Field water capacity was maintained at about 65%. The different salinity levels were applied 50 days after planting. The general principal stated by Boutraa and Sanders(2001) was used for the water treatment application.

**Osmo-regulators treatments:**

A0= control (without osmo-regulators treatment)

A1=1000mg/liter Glutamine

A2=1000mg/liter Glycine betaine

A3= 500mg/L. Glutamine+ 500mg/L. Glycinebetaine.

The three osmo-regulators treatments were sprayed twice during the plant life once after 60 days from sowing and the other four weeks later. The spraying process was foliar and always performed early in the morning. Osmo-regulators materials were provided from Scientific East Group, 7 Kassem Ameen St., Begam-Shoubra, Egypt.

**Experiment design**

This experiment included 12 treatments which were the combination between three saline irrigation treatments (fresh water, 3.13 and 6.25dSm$^{-1}$) and four osmo-regulators treatments [Control (without osmo-regulators), Glutamine, Glycine betaine, and Glutamine+Glycinebetaine treatments]. Treatments were arranged in a randomized complete blocks design with five replicates, different saline irrigation levels were assigned at random in the main plots, while sub-plots were devoted to the different osmo-regulators treatments.

**Measurements:**

Random plant samples of three plants were taken from each treatment at age of 120 days to estimate plant height (cm), number of leaves/plant, stem diameter, leaf area, fresh and dry weights of whole plant. The determination of total soluble solids (TSS) concentration in the cell sap of fresh plant was also estimated by using refracto-meter, the corresponding values of osmotic pressure (Atm) were then obtained from tables given by Gusev (1960).

**Chemical analysis:**

**The photosynthetic pigments** of fresh leaves, chlorophyll a and b,total chlorophyll as well as carotenoids were determined using for such purpose the 4$^{th}$ leaf from the growing point of the plant using the spectrophotometric method recommended by Metzener *et al.*(1965). According to the following equations:

For mg chlorophyll a/g tissue = $(12.7A_{663})$-$2.69(A_{645})$ v/Wx1000.

For mg chlorophyll b/g tissue = $22.9A_{665}$-$4.68A_{645}$ v/Wx1000

total chlorophyll = chlorophyll a + chlorophyll b

Carotenoids content = (1000 $A_{470}$-1.82 chlorophyll a-85.02 chlorophyll b)/198

Samples were collected and dried for 48 h at 70 °C to determine soluble sugars which were extracted following the method of Highkin and Frankel (1962)

**Anatomical studies:** samples were taken for each treatment from the middle of the fifth leaf from apex at 100 days after planting and they were killed and fixed in FAA solution (50 ml 95% ethyl alcohol + 10 ml formalin + 5 ml glacial acetic acid + 35 ml distilled water) for 48 h. Thereafter, samples were washed in 50% ethyl alcohol, dehydrated and cleared in tertiary butyl alcohol series, embedded in paraffin wax of 54–56 °C m.p. Cross sections, 20 µ thick, were cut by a rotary microtome(O'Brien and MeCully, 1981). Adhesive by Haupt's adhesive and stained with the crystal violet–erythrosine combination (Sass, 1961), cleared in carbol xylene and mounted in Canada balsam. The sections observed and documented using an upright light microscope. Sections were microtomed at 15 microns and different areas were estimated in mm$^2$.

**Statistical analyses**

The collected data were subjected to statistical analysis of variance using the normal (F) test and the means were compared using Least Significant Difference (LSD) at 5% level according to Snedecor and Cochran(1980).

## RESULTS AND DISCUSSION

**Effect on growth parameters:**

Data in Table 2 clearly illustrated that, there were significant effects of salinity stress on growth parameters of *Jatropha curcas* L.,on the base of shoot length, leaf number per plant, stem diameter, leaf area, shoot fresh and dry weights. Increasing salinity in irrigation water significantly retarded the elongation of

stems compared with control plants. A negative relationship was obtained for shoot height with increasing salt concentration in irrigation water. The reduction was26.15% at the highest salinity level S2, which revealed the maximum significant reduction in stem height. The reduction in number of leaves and leaf area were only significant at the highest salinity level S2, where the percentages of reduction were48.15% for leaves number and 33.01% for leaf area compared with control plants(Table 2). The same significant reduction was obtained for stem diameter, which significantly reduced by increasing concentration of salt in irrigation water, the reduction reached to34.78% under the S2 treatment as compared to control. A negative relationship was also obtained between shoot fresh and dry weights and salt stress. The percentages of reduction reached to 30.85% and 37.79% for both fresh and dry weights respectively under the highest salinity level S2 compared with control. Similar results were obtained by Jamil *et al.*(2006) who recorded that salinity limited plant growth (shoot and root elongation and shoot and root fresh weights). In addition, Patel, *et al.,* (2010) recorded significant decreases in stem and root lengths, leaf expansion and dry matter accumulation in Jatropha seedlings with increased salinity level. The inhibition in Jatropha growth may be due to the lowering in water potential and turgidity of stressed tissues that caused internal water deficit to plants as recorded by Hishida *et al.,*(2013). However, Munns and Tester(2008)indicated that the inhibition in plant growth by salinity might be due to the bad effects of toxic ions mainly $Na^+$ and $Cl^-$. Kaydan and Okut (2007) and Abdul Qados(2015) obtained similar results; they returned such reductions in growth to decrease in water availability by plant roots, which led to the disturbance in water status of plant's tissues and metabolic processes, leading to reductions in meristematic activity and cell size. It also caused an increment in respiration rate due to the higher energy requirements.

Treated Jatropha plants with different types of osmo-regulators were evaluated for their effect on plant growth parameters in Table (2). Exogenous application of both osmo-regulators singly or in combinations led to improve all growth parameters of jatropha plant significantly compared with untreated plants. It was deduced that glutamine was the most effective treatment in promoting plant growth significantly compared to the control and to other treatments. For plant height and diameter, the highest percentages of increases were assigned to glutamine which reached to 14.06 and 31.25% respectively compared with control, followed by glycine betaine treatment, this result was significant for the two growing seasons. As for leaves number and area, glutamine increased both parameters by 15 and 28.13%respectivelycompared with control, followed by glycine betaine treatment. However, the duel treatment led to a marked decrease in previously mentioned parameters compared with the single treatment but still higher than that of control, except for leaves number. Significant results were obtained by using the three treatments of osmo-regulators for shoot fresh and dry weights, while that of glutamine showed the highest significant means compared with control plants. The increases in fresh and dry weights reached to 57.17 and 89.14% respectively compared to control, followed by glycine betaine treatment. These results were in accordance with that previously reported by Shaddad and Heikal(1982); Thakur and Rai(1985); Hamdia (1987); Cuin and Shabala(2005); Abd El-Samad *et al.*(2011) andEl Sabagh *et al.*(2015). These results may be due to its osmo-protective effect on photosynthetic machinery and regulation of ion homeostasis (Raza *et al.,* 2007).

The interaction effect between salinity and exogenous application of osmo-regulators revealed that there was an increasing trend of reduction in jatropha growth with increasing salinity level. The results also pointed out that using different osmo-regulators treatment was more effective at high salinity level, although glutamine proved to be more effective than the other osmo-regulator treatments in increasing growth parameters of *J. curcas* plant under different salinity treatments compared with their controls; followed by glycine betaine treatment. Observed decrease in growth parameters due to the use of combined treatment of both osmo-regulators compared with single treatment under different salinity levels. This confirmed the study of many authors such Manetas(1990), Silveira *et al.*(2003), Yamada *et al.* (2005) and Abd El-Samad *et al.*(2011).Osmotic and specific ion effects were the most frequently mechanism by which salinity stress inhibited plant growth. Such effects were resulted by reducing water uptake due to nutritional imbalance caused by element antagonism or toxicity (Najafi*et al.*,2010). The regulatory effect of amino acids application may be through their effect on gibberellins biosynthesis (Walter and Nawacke, 1978). In addition, amino acids couldplay an important role inplant metabolism and protein assimilation, which was important for cell formation, and consequently increase in fresh and dry weights. Our result here was in agreement with that result recorded by Cuin and Shabala (2005)who recorded that the role of compatible solutes was not limited to osmotic adjustment function only, but also to its osmo-protective effect. Such as maintaining cytosolic Kions homeostasis by preventing NaCl induced K ion leakage from the cell.This result was in accordance with that of Manetas, (1990); Silveira *et al.,*(2003); and Yamada *et al.,*(2005).

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**Table 2: Effect of salinity stress and treatment with osmo-regulators on growth parameters of *Jatropha curcas* L.**

| Treatments | | Plant height (cm) | No. of leaves/plant | Stem diameter (cm) | Leaf area ($cm^2$) | F.W/plant (g) | D.W/plant (g) |
|---|---|---|---|---|---|---|---|
| Salinity stress | | | | | | | |
| S0 | | 80 | 27 | 2.3 | 112.86 | 261.75 | 71.52 |
| S1 | | 70 | 23 | 1.8 | 116.08 | 224.25 | 64.38 |
| S2 | | 59 | 14 | 1.5 | 75.60 | 181.00 | 44.49 |
| $LSD_{0.05}$ | | 3.12 | 4.62 | 0.08 | 4.41 | 7.11 | 3.77 |
| Osmo-regulator treatments | | | | | | | |
| A0 | | 64 | 20 | 1.6 | 89.66 | 169.67 | 40.16 |
| A1 | | 73 | 23 | 2.1 | 114.88 | 266.67 | 75.96 |
| A2 | | 70 | 22 | 2.0 | 106.56 | 236.00 | 63.79 |
| A3 | | 69 | 20 | 1.7 | 94.95 | 217.00 | 60.61 |
| $LSD_{0.05}$ | | 2.42 | 3.51 | 0.11 | 5.24 | 6.52 | 2.97 |
| Salinity stress X Osmo-regulator treatments | | | | | | | |
| S0 | A0 | 75 | 26 | 2.1 | 88.34 | 198 | 50.35 |
| | A1 | 84 | 29 | 2.5 | 131.3 | 333 | 89.33 |
| | A2 | 80 | 27 | 2.4 | 130.9 | 266 | 75.36 |
| | A3 | 79 | 26 | 2.2 | 100.9 | 250 | 71.02 |
| S1 | A0 | 63 | 21 | 1.5 | 112.21 | 191 | 47.13 |
| | A1 | 74 | 25 | 2.1 | 119.31 | 251 | 74.22 |
| | A2 | 72 | 24 | 1.9 | 117.21 | 233 | 70.21 |
| | A3 | 70 | 21 | 1.7 | 115.60 | 222 | 65.96 |
| S2 | A0 | 55 | 13 | 1.1 | 68.42 | 120 | 22.99 |
| | A1 | 62 | 16 | 1.8 | 94.03 | 216 | 64.32 |
| | A2 | 59 | 14 | 1.6 | 71.57 | 209 | 45.80 |
| | A3 | 58 | 12 | 1.3 | 68.36 | 179 | 44.85 |
| $LSD_{0.05}$ | | 3.88 | 5.11 | 0.33 | 6.14 | 4.98 | 3.68 |

S : Salinity, S0: fresh water, S1 : 0.31 $dSm^{-1}$ S2 : 6.25 $dSm^{-1}$

A : osmo-regulators, A0 : No osmo-regulators , A1 : glutamine, A2 : glycine betaine, A3 : glutamine+ glycine betaine.

**Anatomical modifications associated with high salinity:**

The characteristics for control and treated plants were shown as microphotographs illustrated inFigs.1-8.Salinity had a marked effect on leaf anatomy; where high salinity level caused a significant reduction in leaf area, as we mentioned before in Table 2. Cross sections of J. curcas leaf were analyzed to assess the effects of high salinity level S2 and different osmo-regulators on the atomical adaptations of *J. curcas* plant. By examination, the transverse sections, which were taken from leaf of control and high salinity treated plants; the figures showed the following parts: 1) Upper epidermis layer, 2) Mesophyll layer, 3) Lower epidermis layer (Fig 1). The upper epidermis was the outermost layer, one cell thick, covered with cuticle. Mesophyll layer was differentiated into two parts, the palisade tissue towards upper epidermis layer, and the spongy tissue towards the lower epidermis layer. The palisade tissue had 2-3 layers of cells and was below the epidermis layer. Spongy tissue followed the palisade layer, which were 2-3 cell layers thick. Both these tissues had chloroplast and were photosynthetic. Comparison of the thickness (in mm) of palisade tissue in both treatments was observed in (Table 5). This tissue had chloroplast and hence photosynthetic activity. It was evident that the palisade tissue showed marked increase in thickness with increase in salinity compared to control. As for spongy tissue; this was in continuation of palisade tissue layer and was photosynthetic due to the presence of chloroplast. The thickness of this tissue was more under high salinity level S2and densely arranged compared with plants treated with fresh water S0.Thus, the thickness of photosynthetic spongy tissue increased with increase in salinity stress. Therefore, the thickness of the photosynthetic mesophyll tissue increased with increasing salinity level as compared with control plants. Enhanced spongy tissue in salt treated plants of *J. curcas* helped in maintaining leaf water content and turgor (Parida *et al.,* 2016).Mesophyll

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thickness also increased with salinity due to an increase in thickness of palisade layer and an increased thickness of spongy layer (Longstreth and Nobel.,1979). These results were in accordance with those reported by David and Park (1979) on cotton and atriplex and Hussein *et al.*(2012) on *Jatropha curcas.*

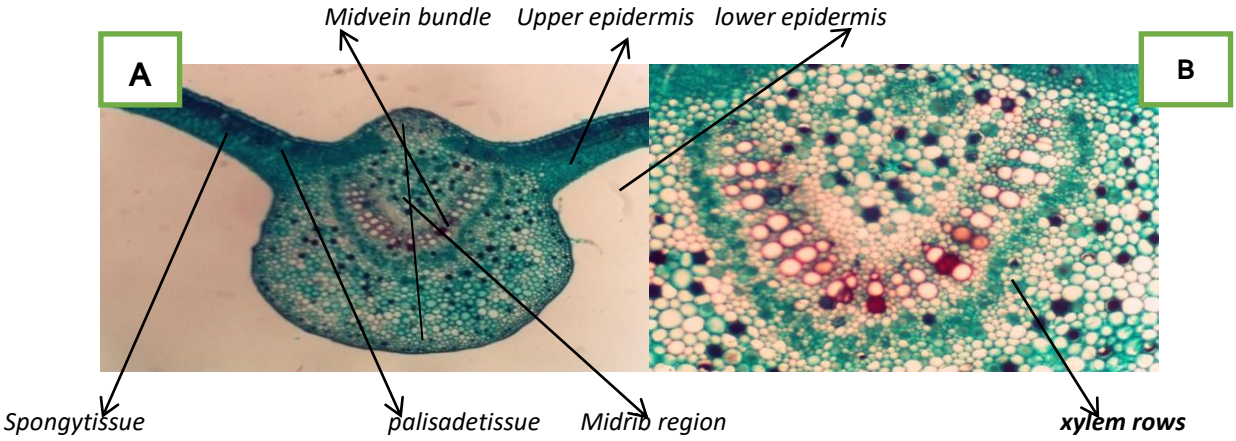


**Fig 1: control treatment (fresh water without osmo-regulators)**

**Fig 2: control (fresh water) + Glutamine**

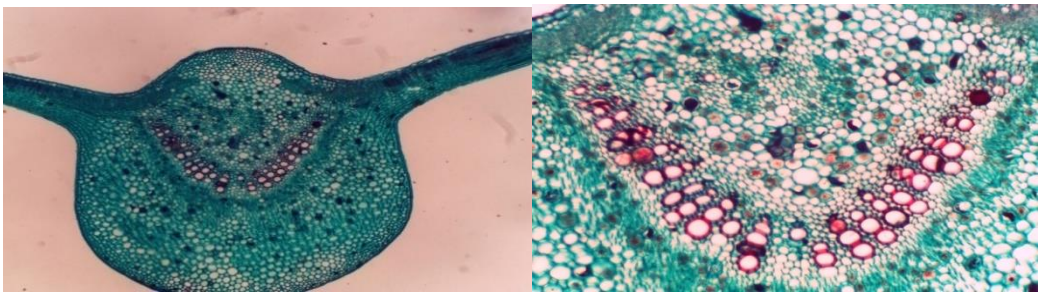

**Fig 3: control (fresh water) + glycine betaine**

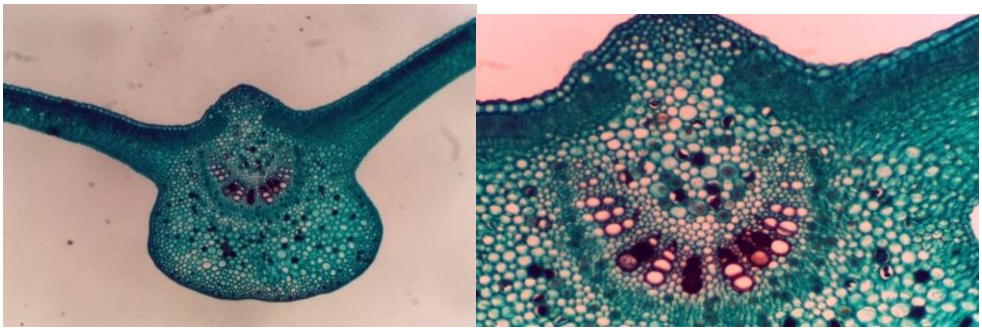

**Fig 4: control (fresh water)+Glutamine and Glycine**

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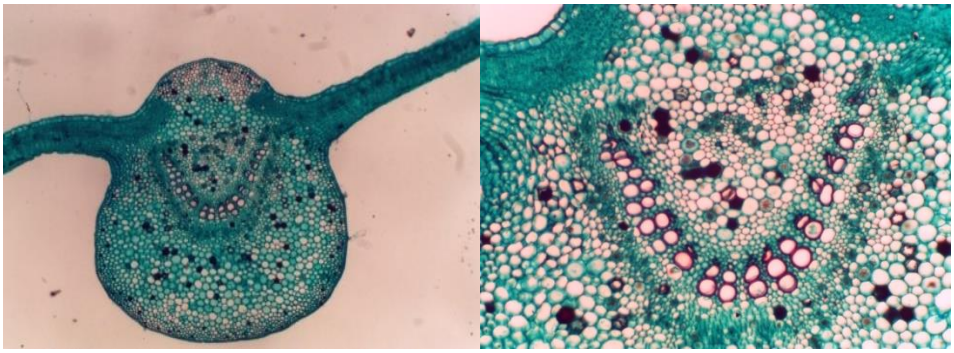

Fig 5: high salinity level S2 without osmo-regulators

Fig 6: high salinity level S2+ Glutamine

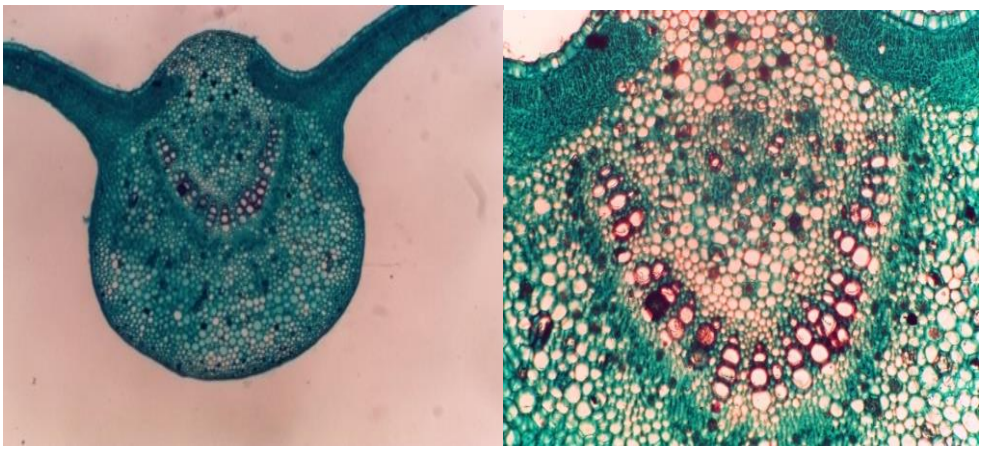

Fig 7: high salinity level S2+ Glycine

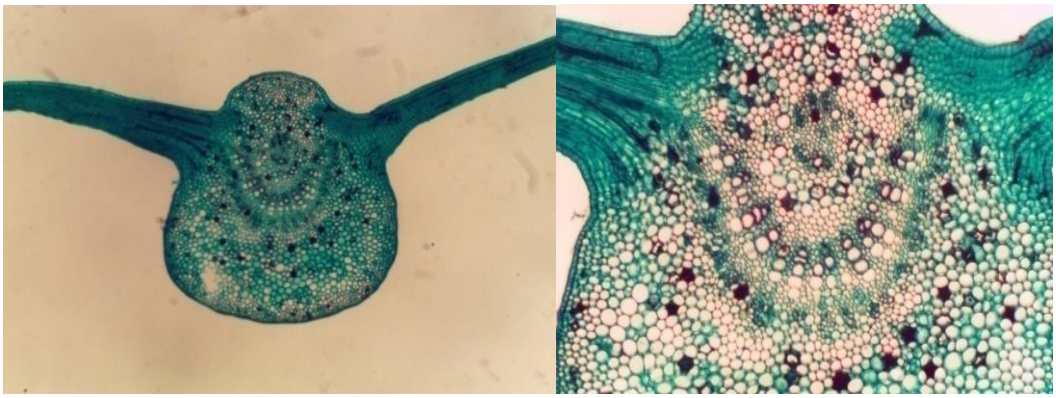

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**Fig 8: high salinity level S2+ Glutamine andGlycine**

**A=** Light Microphotograph showing transverse section through the blade of the fourth leaf developed on the main stem of *Jotropha plants*(x = 10) (Bar=0.1 ml).
**B**= Light Microphotograph showing transverse sections through the blade of the fourth leaf developed on the main stem of *Jotropha plants*. The section shows focus on vascular bundle ( number of vessels andnumber of xylem rows). (x40)(Bar=0.05ml).

**Anatomical modifications in *Jatropha curcas* L. leaf as response to salinity stress**

The data revealed also that there was an increment in midrib and lamina thicknesses under the highest salinity level S2, compared with control treatment. The increase in lamina thickness may be due to the change in all cell types or perhaps due to change in epidermal thickness or vesicular bundle thickness, mesophyll thickness or bundle sheath (Kemp and Cunningham, 1980). Increase in lamina thickness could be also due to the mesophyll bulk rather than water storage in hypodermal tissue. Thus, salinity induced increase in leaf thickness due to water storage probably to decrease the effect of salinity(Nandy *et al.,* 2007). Similar effect on leaf thickness had been reported previously by Meirand Mayber(1967), Wignarajah*et al.* (1975) and Hussein *et al.*(2012). In addition, Vascular bundle area (Width and length) was maximum under control conditions compared with that of high salinity level. Vascular bundle area seemed to be directly related to efficient transport of water and nutrients from the soil and these could be of greater importance under stress conditions (Awasthi and Pathak, 1999, and Ali*et al.,*2009). The data also revealed an observed reduction in number of vessels under the highest salinity level S2 compared to control. Moreover, greater number of xylem rows were obtained under high salinity level S2 compared with control.

**Table 3: Effect of salinity stress and treatment with osmo-regulators on leaf anatomy of *Jatropha curcas* L.**

| treatments | Spongy layer Thickness mm | palsied layer thickness mm | Mesophyll layer thickness mm | vascular bundle Width mm | vascular bundle Length mm | No. of Vessels | No. of xylem rows | Lamina Thickness mm | Midrib Thickness mm |
|---|---|---|---|---|---|---|---|---|---|
| S0 XA0 | 27.5 | 20 | 52 | 144 | 164 | 53 | 19 | 62 | 320 |
| S0XA1 | 28.5 | 23 | 69 | 158 | 190 | 75 | 24 | 76 | 344 |
| S0XA2 | 31 | 27 | 67 | 156 | 200 | 62 | 21 | 70 | 340 |
| S0XA3 | 25 | 20 | 53 | 90 | 110 | 35 | 13 | 90 | 264 |
| S2XA0 | 30 | 23 | 63 | 120 | 140 | 50 | 21 | 80 | 334 |
| S2XA1 | 33.5 | 29 | 107 | 122 | 158 | 60 | 24 | 100 | 400 |
| S2XA2 | 31.5 | 30 | 70 | 160 | 180 | 60 | 22 | 90 | 316 |
| S2XA3 | 31.5 | 32 | 76 | 100 | 120 | 50 | 17 | 60 | 260 |

S : Salinity, S0: fresh water, S1 : 0.31 $dSm^{-1}$ S2 : 6.25 $dSm^{-1}$
A : osmo-regulatores, A0 : No osmo-regulators , A1 : glutamine, A2 : glycine betaine, A3 : glutamine+ glycine betaine.

Our results were generally in accordance with those obtained by Wignarajak *et al.* (1975)on beans, Reda *et al.* (2000) on leucaena, and Boghdady (2009) on mung bean.

In addition, it can be obviously noticed that single application of glutamine or glycine betaine increased mostly the thickness of both the mid vein and lamina of *J. curcas* leaf compared with control, while dual treatment revealed mostly bad effect on both parameters. The increases in the lamina thickness were accompanied by increases in the thickness of the palisade and spongy tissues and there for increase in mesophyll tissue thickness compared with the control. While, dual treatment was more effective under S2

treatment compared to control. Likewise, the average number of vessels and xylem rows of the mid vein increased because of osmo-regulators treatments. In addition, vascular bundles increased in size because of the single treatment of glutamine or glycine betaine. The increment was primarily attributed to the increase in the width and length of vascular bundles compared with the control, also dual application of both treatments caused sharp decrease on these parameters. These results were generally in agreement with those of Boghdady(2009)on mung bean and with Hussein *et al.* (2012) on Jatropha. The results also indicated that osmo-regulator treatments enhanced most of the histological characteristics of stressed leaf, and these results suggested that the foliar application of both glutamine or glycine had the ability to decrease the deleterious effect of salinity on the anatomical structure of *J. curcas* leaves.

**Effect on photosynthetic pigments:**

Salinity stress increased significantly chlorophyll a, chlorophyll b, and total pigment contents of *J. curcas* leaves (Table 4). A positive relationship between salt concentration and pigment concentrations compared with control. The percentage of increase reached 42.27% for Chla, 71.81% for Chlb and60.39% for chla+bunder the highest salinity level (S2), which revealed the maximum increment in photosynthetic pigments compared with control. While, opposite trend was obtained for carotenoids content; where salinity stress significantly reduced carotenoids content of *J. curcas* leaves as compared to control. These reductions were maximum at 6.25 $dSm^{-1}$; where the percent of reduction reached to 38.01% compared to the control. Moreover, carotenoids considered as an accessory pigment (absorb sun light only when chla and chlb cannot absorb sun light) (Khalil and Yousef, 2014).The increase in Chla, Chlb and total chlorophyll may be due to the increase in thickness of palisade and spongy tissues of *J. curcas* leaves. Which were the chlorenchymatous mesophyll tissue, which contains numerous chloroplasts and considered principal site for photosynthesis (Xu *et al*., 2014) or may be due to reduced leaf water content due to the effect of salinity (Yuwono *et al*., 2005). These results were in accordance with the findings of Petersen *et al.* (1998)and James *et al.*(1999).

Foliar spray of osmo-regulator to *J. curcas* plant enhanced photosynthetic pigment by increasing chlorophyll *a*, chlorophyll *b*, total pigment, and carotenoids content significantly as compared to control(table 4). The increase was more significant when using glutamine treatment then glycine betaine treatment. The percentage of increase under glutamine treatment reached to 13.5 % for Chla, 71.89% for Chlb, 47% for Chla+Chlb and 71.22%for carotenoids content. Furthermore, Awad *et al.* (2007) and Sadak *et al.*(2015) reported similar results. This increment in chlorophyll concentration could be attributed to the availability of higher levels of amino acids in treated plants that induced an increase in chlorophyll content (Awad *et al.,* 2007). Makela *et al.*(1998b)indicated that Glycine betaine caused an increase in net photosynthesis, stomatal conductance, decreased rate of photorespiration, and induced more efficient gas exchange.

The combined effect of salinity and osmo-regulators was evaluated also in table 4. Osmo-regulators treatments caused stimulatory effects on chlorophyll *a*, chlorophyll *b*, total pigment, as well as carotenoids concentration compared to control under both saline and non-saline (control) conditions; especially in plants subjected to sea salt stress in both seasons. It was clear from the data that S2XA1 treatment showed the highest significant values of chlorophyll *a*, chlorophyll *b*, total pigment compared with other treatments and their control in both seasons. While S0XA1 treatment showed the highest significant means for carotenoids, content compared with other treatments and their control in both seasons. The efficiency of the photosynthetic apparatus was stimulated by osmo-regulators treatment due to increase in the biosynthesis of osmotic solutes under salinity stress that, enhanced water flow into different plant organs and tissues due to increase in osmotic pressure. So osmo-regulators treatment could directly or indirectly influence the physiological activities of the plant such as photosynthesis and its pigments. Raza *et al.,*(2007) also reported that osmo-protectants promotes plant growth and yield under normal or stress conditions due to its osmo-protective effect on photosynthetic machinery and regulation of ion homeostasis.

**Table 4: Effect of salinity stress and treatment with osmo-regulators on photosynthetic pigments of *Jatropha curcas* L. leaves.**

| Treatments | Chla | Chlb | Chla+chlb | Carotenoids |
|---|---|---|---|---|
| Salinity stress | | | | |
| S0 | 4.85 | 7.70 | 12.55 | 3.13 |
| S1 | 5.93 | 12.45 | 18.38 | 2.81 |

| | | | | | |
|---|---|---|---|---|---|
| S2 | | 6.90 | 13.23 | 20.13 | 1.94 |
| $LSD_{0.05}$ | | 0.21 | 1.04 | 2.01 | 0.09 |
| Osmo-regulator treatment | | | | | |
| A0 | | 5.69 | 7.65 | 13.34 | 2.05 |
| A1 | | 6.46 | 13.15 | 19.61 | 3.51 |
| A2 | | 5.79 | 12.13 | 17.92 | 2.75 |
| A3 | | 5.63 | 11.57 | 17.20 | 2.19 |
| $LSD_{0.05}$ | | 0.48 | 0.89 | 1.01 | 0.42 |
| Salinity stressX Osmo-regulator treatment | | | | | |
| S0 | A0 | 4.96 | 5.08 | 10.04 | 2.41 |
| | A1 | 4.90 | 9.51 | 14.41 | 4.85 |
| | A2 | 4.72 | 9.12 | 13.84 | 2.92 |
| | A3 | 4.80 | 7.10 | 11.90 | 2.32 |
| S1 | A0 | 5.71 | 8.80 | 14.50 | 2.11 |
| | A1 | 6.96 | 14.59 | 21.55 | 3.85 |
| | A2 | 5.95 | 12.86 | 18.81 | 3.04 |
| | A3 | 5.11 | 13.54 | 18.65 | 2.24 |
| S2 | A0 | 6.41 | 9.08 | 15.49 | 1.62 |
| | A1 | 7.51 | 15.35 | 22.86 | 1.83 |
| | A2 | 6.69 | 14.41 | 21.10 | 2.29 |
| | A3 | 6.99 | 14.07 | 21.06 | 2.01 |
| $LSD_{0.05}$ | | 0.67 | 1.10 | 3.12 | 0.78 |

S : Salinity, S0: fresh water, S1 : 0.31 $dSm^{-1}$ S2 : 6.25 $dSm^{-1}$
A : osmo-regulator, A0 : No osmo-regulators , A1 : glutamine, A2 : glycine betaine, A3 : glutamine+ glycine betaine.

**Effect onosmotic pressure:**

Data in Table 5illustrated that the salinity stress induced significant increases in the values of TSS and osmotic pressure compared with control in both seasons. Similar effects of salinity on TSS and osmotic pressure was observed by Munns *et al.*(2002); Ono *et al.*(2003); Marayama *et al.*(2004); Najafi(2010);Abd El-Samad *et al.*(2011) and Khalil and Abou Leila (2016). The observed increase in TSS and osmotic pressure as a result of increasing salinity level may be due to the accumulation of osmotic agents as amino acids especially proline and organic acids or sugars for osmotic adjustment function to maintain its turgidity (Abdel-Haleem 1985). Moreover, Marayama *et al.*(2004) indicated that,when plants exposed to environmental stress, such as drought or salinity, they activate various metabolic and defenses mechanisms to survive, one of them was osmotic adjustment.

Exogenous application of different osmo-protectants treatments on *J. curcas* plant caused significant decreases in TSS and osmotic pressure means compared to control plants. The lowest significant values were recorded in single treatment with glutamine, followed by glycine betaine compared to control. The present findings were in complete agreement with those findings of Sudhakar *et al.,*(1993);Jagendorf and Takabe(2001);Giridarakumar *et al.,*(2003); Sawahel,(2004)and Ranganayakulu *et al*(2013).These results may be due to the ability of osmo-protectants to lower the osmotic potential and increasing the relative water content of plant tissues (Kazemi,2013). It also may due to that; amino acids treatments stimulated the biosynthesis of osmotic solutes under salinity stress. These osmolytes could enhance water flow into different plant organs and tissues by affecting the osmotic pressure of the cytoplasm. This might explain why amino acids could alleviate the effect of imposed salt stress, either via conferring desiccation resistance to plant cells or by osmotic adjustment (Sadak *et al.,* 2015).

The interaction between different salinity levels and different osmo-protectants treatments had significant effects on TSS and osmotic pressure values in both growing seasons (Tale 5).Using different osmo-protectants treatments was not so effective in changing the TSS and osmotic pressure values in leaves of *J. curcas* plant under fresh water treatment (control). Furthermore, there were observed decreases in leaves TSS and osmotic pressure means due to the use of different osmo-protectants treatments under different salinity

levels compared with their control. Moreover, single osmo-protectant treatment proved to be more effective in lowering the values of TSS and osmotic pressure than combined treatment, especially glutamine treatment. Several investigators (Subbarao *et al.,* 2001; Giridarakumar *et al.,* 2003) reported thatglycine betaine was one of the quaternary ammonium compounds which was considered as an effective compatible solute that accumulated in the chloroplasts of certain plants when exposed to environmental stresses, such as drought or salinity. It might play a major role in maintaining intracellular osmotic equilibrium during stress conditions. In addition, glutamine seemed to be the predominant pathway under stress conditions, due to the repressed salt-stress ornithine omega-aminotransferase expression (the enzyme responsible for synthesis of Proline from Ornithine), induced the synthesis of Proline from Glutamine (Delauney and Verma, 1993 and Delauney *et al.,* 1993).

**Effect on total soluble sugars content:**

Increasing salinity level resulted in significant increases in total soluble sugar percentage, being more pronounced under the severe salinity stress S2 treatment, compared with control in both seasons (Table 5). These results were in line with that recorded by Devitt *et al.*(1987); Hernandez *et al.,*(2000); Rai *et al.,*(2003); Yamad *et al.,*(2005) and Abd El-Samad *et al.,*(2011). The accumulation of compatible solutes such as soluble sugar might help to maintain the relatively high turgedity (RWC), which was necessary in osmo regulation needed for plant growth and cellular functions(Abd El-Samad *et al.,* 2011). Furthermore, Sacher and Staples(1985) reported that increasing sugar levels relative tocontrol in salt stressed plants might contribute to the turgor maintenance.

**Table 5: Effect of salinity stress and treatment with osmo-regulators on total soluble solids, osmotic pressure and total soluble sugars of *Jatropha curcas* L. leaves.**

| Treatments | | TSS | Osmotic pressure (Atm) | Total soluble sugars% |
|---|---|---|---|---|
| Salinity | | | | |
| S0 | | 7.64 | 6.09 | 49.59 |
| S1 | | 7.83 | 6.27 | 56.44 |
| S2 | | 8.45 | 6.82 | 64.28 |
| $LSD_{0.05}$ | | 0.08 | 0.02 | 2.66 |
| Osmo-regulators | | | | |
| A0 | | 8.16 | 6.56 | 51.27 |
| A1 | | 7.89 | 6.31 | 62.98 |
| A2 | | 7.91 | 6.33 | 54.73 |
| A3 | | 7.94 | 6.36 | 58.10 |
| $LSD_{0.05}$ | | 0.04 | 0.04 | 2.54 |
| Salinity X Osmo-regulators | | | | |
| S0 | A0 | 7.77 | 6.21 | 44.35 |
| | A1 | 7.60 | 6.05 | 55.95 |
| | A2 | 7.60 | 6.05 | 45.87 |
| | A3 | 7.60 | 6.05 | 52.18 |
| S1 | A0 | 7.90 | 6.33 | 53.55 |
| | A1 | 7.77 | 6.21 | 62.83 |
| | A2 | 7.83 | 6.27 | 52.53 |
| | A3 | 7.83 | 6.27 | 56.83 |
| S2 | A0 | 8.80 | 7.14 | 55.90 |
| | A1 | 8.30 | 6.68 | 70.17 |
| | A2 | 8.30 | 6.68 | 65.78 |
| | A3 | 8.40 | 6.77 | 65.28 |
| $LSD_{0.05}$ | | 0.12 | 0.23 | 3.19 |

S : Salinity, S0: fresh water, S1 : 0.31 $dSm^{-1}$ S2 : 6.25 $dSm^{-1}$

A : osmo-regulators, A0 : No osmo-regulators , A1 : glutamine, A2 : glycine betaine, A3 : glutamine+ glycine betaine.

Moreover, foliar spray of osmo-regulators significantly improved total soluble sugar percentage relative to control. These increments reached their maximum values in plants treated with glutamine, followed by combined treatment of glutamine+glycine betaine(A3 treatment) compared with control plants. The overall improvement due to application of osmo-regulators may be due to the promoted effects of amino acids treatment that improve cell-internal function as osmo-regulatory, which increase the accumulation of cellular osmotic components (Ranganayakulu *et al.,*2013).Moreover, Neuberg *et al.,*(2010) pointed out that glutamine significantly increased plant growth elements, soluble sugars, sulphur compounds, soluble phenols, free amino acids, photosynthetic pigments of leaves as well as quantity and quality of onion bulbs.

The interaction between salinity stress and different osmo-regulators treatments figured out that all osmo-regulators treatments increased total soluble sugar percentage significantly under control water and under different salinity levels. The maximum increases in total soluble sugar percentage were observed under the effect of interaction between the highest salinity level and glutamine treatment S2XA1 treatment compared with the other treatments. The present findings were in complete agreement with the finding of Hounsome *et al.,*(2008), Neuber*get al.,*(2010) and Amin *et al.,*(2011).These increases in total soluble sugar percentage because of osmo-regulators treatments may be due to the close relationship between the effect of amino acids and the stimulation of the photosynthetic output (soluble sugars, polysaccharides, and total carbohydrates) of the plant (Abd El-Monem , 2007).

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