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Role Of Bioregulator On Proline, Proteins And Soluble Sugar Accumulation In Organs Of Okra Under Salinity Stress

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Abstract: *Okra is an important vegetable grown in tropical and sub-tropical regions. Here, we study its response to salinity stress. Seeds of 47-4 and LD88 genotypes were pre-soaked with indole acetic acid (0.4, 0.5 and 0.6mM) and control in distilled water. The seeds were grown in polyethylene bags under different levels of salinity (50, 100, 150 and 200 mMNaCl).Experiment was conducted to study the interactive effect of salinity and bioregulator on growth and accumulation of solutes (proline, salt stress proteins and soluble sugar). Salinity stress in all levels increased proline content in the two genotypes in increasing order in the absence of indole acetic acid.Values of soluble sugars and salt stress proteins were decreased by severe salt stress in the organs of the two genotypes whileinteractions of salinity with IAA treatments particularly at 0.4 and 0.5 mM modulate the negative effect on the plants and improved these values as compared to treated control group. However, in the two studied organs of stressed okra, fruit showed more soluble sugar accumulation than leaf in both genotypes. Thus, it is of noteworthy that indole acetic acid treatments at 0.4 and 0.5 mM serve as promising concentrations to alleviate the effects of salinity stress on okra plant, which may be due to reduction in reactive oxygen species at those concentrations. IAA could also be used to enhance plant growth as well as accumulation of non-toxic metabolites particularly under mild and moderate salinity levels.*

Keywords:*Distilled water, Indole acetic acid, Okra, Proline, Protein, Salinity, Sugar*

I. Introduction

All plants in one way or the other throughout their life span are subjected to varieties of stresses. Plant responds in different ways to stress based on the plant species as well as the source of the stress. When a certain limit level is reached, plant will eventually die. The two most important environmental factors that currently reduce plant productivity are drought and salinity (Tester and Davenport, 2003), and the reactions of these stresses are similar in plant due to water stress. These environmental factors affect plants in a tremendous ways, for example, biotic factors decrease crop yields by less than 10%, whereas intense environmental problems can be responsible for up to 65% reduction in yield (Serrano, 1999). Salt stress constitutes a major challenge to plants; globally it limits agricultural productivity (Parida and Das, 2004), salt tolerance is relevant in vegetables because of the cash value of crops (Shannon and Grieve, 1999). As a result of poor irrigation practices on majority of lands, impact of salinity is becoming more important (Winicov, 1998). Ions toxicity, osmotic and nutrition imbalance are the major constraints of plants under salt stress (Lauchli and Epstein, 1990). At low salt concentrations, yields are mildly affected or not at all, but increase in salt concentrations resulted in poor yields (Maggio *et al*., 2001). Salinity affected physiological and biochemical processes, such as translocation, ion uptake, respiration, photosynthesis, carbohydrates, nutrient metabolism and hormones (Farooq*et al*., 2009), and bring about plant growth retardations, which can eventually lead to death. Biochemical and molecular studies of salt stress responses in plants showed significant increases of reactive oxygen species (ROS), including singlet oxygen ($^1O_2$), superoxide (O_2^-), hydroxyl radical (OH •) and hydrogen peroxide (H_2O_2) (Tanou*et al*. 2009 ; Ahmad *et al*. 2010a, 2012 ; Ahmad and Umar 2011). However, the negative effect of salt stress on plants depends on the time of exposure and concentration of salinity, plant genotypes as well as environmental factors. Okra is a member of Malvaceae, originated in tropical Africa and grown in Mediterranean region; production of this crop is limited because of water and soil salinity. Several biochemical pathways determine the tolerance to salt stress. These pathways play the role in protection of protoplast functions, maintenance of ion homeostasis and control of ion and water flux. These methods include synthesis of osmotic adjustment, specific protein and free radical enzymes (Parida and Dos, 2005).

In recent years exogenous protectant such as osmoprotectants (proline, glycinebetaine, trehalose, soluble sugar etc.), plant hormone (gibberellic acids, jasmonic acids, indole acetic acid, salicylic acid, etc.), antioxidants (ascorbic acid, glutathione, tocopherol, etc.), signaling molecules (nitric oxide, hydrogen peroxide, etc.), polyamines (spermidine, spermine, putrescine), trace elements (selenium, silicon, etc.) have been found effective in modulating damage in plant induced by salt stress (Hoque*et al*. 2007 ; Ahmad *et al*. 2010a, 2012 ; Azzedine*et al*. 2011 ; Hasanuzzaman*et al*. 2011a, b ; Hayat and Ahmad 2011 ; Hossain*et al*. 2011 ; Poor *et al*. 2011). Most organic compatible solutes in the cytoplasm and organelles play a role in osmotic adjustment in the vacuole. Proline accumulated under salt stress performs the positive role in the adaptation of cells to salt and

water stress (Kaviani, 2008). Proline plays an important role in protein accumulation and in cell adaptation to salinity stress (El-Enany, 1995), thus accumulation of proline in plant may be related to osmotic and saline stress tolerance (Watanabe *et al*., 2000). Therefore, this present study was designed with the objective to investigate the effect of indole acetic acid on negative effects of saline, as well as on accumulations of compatible solutes in okra plant, and also to determine the best concentration of indole acetic acid for better accumulation of metabolic constituents in the okra plant.

II. Materials and Methods

2.1 Plant materials and growth conditions

Screen house experiments were carried out at the National Horticultural Research Institute, Ibadan, Oyo state, Nigeria. Seeds were collected from genetic resource laboratory (Product Development Programme). Planting was done in polyethylene bags filled with 10kg sandy soil with $_{P}$H 7.10, Exch. Acidity 0.34, Clay (%) 12.30, Silt (%) 13.90, Sand (%) 65.40, Organic Carbon (g/Kg) 47.32, Nitrogen (g/Kg) 2.53, Phosphorous (mg/Kg) 20.00, Potassium (cmol/Kg) 1.33, Sodium (cmol/Kg) 0.89, Calcium (cmol/Kg) 45.65, Magnesium (cmol/Kg) 13.34. The seeds of the two genotypes were surface sterilized with 1% sodium hypochloride solution (NaOCl) for 2 minutes, and finally rinsed with double distilled water before soaking in various pre-treatment concentrations of 0.4 mM, 0.5 mM and 0.6 mM of Indole acetic acid and control seeds in distilled water for 12 hours in the dark at 25^{0C}. Thereafter, the solutions were decanted off and seeds washed 2-3 times with double distilled water. The seeds were vacuum dried for 1 hour. The seeds were then grown in the soil without NaCl (control) and under salinity levels corresponding to osmotic potential of NaCl solution of 50, 100, 150, and 200 mM. Saline solutions were added to the soil in such a way that the soil acquired the assigned salinity levels at field capacity. All 60 pots for each genotype (3 treatments X 5 levels X 3 replicates) were irrigated with normal tap water on weekly basis to achieve soil water field capacity for the period of eight weeks. Treatment sets were as follow:

I. Control and salt treatments: (reference group).
a. (0 NaCL). Control
b. 50 mMNaCl.
c. 100 mMNaCl.
d. 150 mMNaCl.
e. 200 mMNaCL
II. Indole acetic acid and salt treatments (IAA treatment):
0.4mM IAA+ 0 mMNaCL. 0.5mM IAA+ 0mM NaCl. 0.6mM IAA+0mM NaCl
0.4mM IAA+50 mMNaCL .0.5mM IAA + 50mM NaCl.0.6mM IAA + 50mM NaCl.
0.4mM IAA+100 mMNaCL. 0.5mM IAA +100mM NaCl 0.6mM IAA + 100mM NaCl.
0.4mM IAA+150 mMNaCL. 0.5mM IAA +150mM NaCl.0.6mM IAA +150mM NaCl.
0.4mM IAA+200 mMNaCL.0.5mM IAA + 200mM NaCl. 0.6Mm IAA + 200mM NaCl.

After eight weeks of germination, fruits of each plant of each group were collected to investigate the biochemical changes in proline, protein and soluble sugar accumulation in leaf and fruit of the two genotypes.

2.2 Proline determination

Concentrations of free proline were determined according to Bates *et al*. (1973). Five-hundred milligrams of the dried leaf and fruit samples were dissolved in 10 ml of 3% (v/v) aqueous sulfosalicylic acid. The homogenate was filtered through Whatmanno 41 filter paper. The filtrate was acidified with glacial acetic acid and ninhydrin (1 ml each) and was heated in water bath at100^{0C} for 1 h. The mixture was extracted with 5 ml toluene and the upper (toluene) phase decanted into a glass cuvette and the absorbance was measured at 520 nm. Proline concentrations were calculated using proline standards (0–50 µg/ml).

2.3 Soluble protein determination .

Soluble protein accumulation was analyzed by the method of Lowery *et al*., (1951) using Folin-Ciocalteau reagent. Five-hundred milligrams of the dried leaf and fruit samples were weighted and digested by hot ethanol 80% two times, each on 10 mL and the extract diluted to 50 mL by double distilled water. The absorbance of blue color was read in 660nm by spectrophotometer machine (Pharmaspec UV-1700 model). The amount of soluble protein was calculated from bovine serum albumin standard curve.

2.4 Soluble sugar determination

Soluble sugars were determined using colorimetric method described by Dubois *et al*. (1956). Glucose was applies as a standard.

2.5 Statistical analysis

The factorial experimental design with two varieties, two genotypes and four salinity levels were arranged in a completely randomized design (CRD) with three replications and the data were analyzed using the software package, SAS windows and the mean separation by LSD0.05.

III. Results

The result on table 1 showed that as the level of salinity increased the proline accumulation increased in the leaf and fruit of the two genotypes as compared to the control group. However, in both genotypes (LD88 and 47-4), the two studied organs of salinity stressed okra; leaf and fruit showed decreased concentration of protein accumulation with increased salinity levels. The least accumulation of protein in the leaf and fruit of both genotypes were seen at 200mMNaCl (Figure 1 and 2).

Figure 3 and figure 4 showed the interaction between the bioregulator and the salinity on protein content. The interaction in 47-4 showed a progressive increase in protein content in the fruit and leaf especially with IAA treated groups at high and mild salinity levels (0.4 mM IAA at 100 mMNaCl, 0.5 mM IAA at 50 mMNaCl and 0.6 mM IAA at 200 mMNaCl) when compared with the control groups (Figure 3). While in LD88, increase in protein accumulation was seen in the groups treated with 0.4 mM IAA at 100 and 150 mMNaCl, but no significant effect recorded in leaf of LD 88 (figure 4). Among the two studied organs of salinity stressed okra in the two genotypes, 47-4genotype showed more protein accumulation than LD88.

In 47-4 genotype, salinity reduced soluble sugar content in both leaf and fruit as compared to the control group (figure 5).In LD88 genotype, accumulation of soluble sugar was affected only in the fruit, but as the salinity levels increased soluble sugar accumulation increased in the leaf and the highest soluble sugar accumulation was seen in the group treated with 200 Mm NaCl (figure 6). Figure 7 and figure 8 showed the interaction between the bioregulator and the salinity on reducing sugar accumulation. Similarly, the interaction in 47-4 showed a progressive increased in soluble sugar accumulation in the fruit especially with the groups treated with 0.4 mM and 0.5 mM IAA at high salinity levels of 100, 150, and 200 mMNaCl when compared with the control group, but interaction showed little or no effect on reducing sugar content in leaf (figure 7). In the same vein, in LD88, increased soluble sugar accumulation was seen in the fruit of groups treated with 0.4mM IAA at 50, 100, 150, and 200mM NaCl (figure 8). Among the two studied organs of salinity stressed, fruit showed more soluble sugar accumulation than leaf in the two genotypes.

Figure 9 and 10 showed the effect of bioregulator on protein content. The result revealed that in genotype 47-4 protein accumulation in the leaf of group treated with 0.4mM IAA was significantly increased as compared to the control group (Figure 9).But in the genotype LD88 increased level of protein accumulation in fruit was seen in group treated with 0.5mM IAA when compared with control group (Figure 10).The data in Figure 11 showed increased in soluble sugar accumulation in the fruit of the group treated with 0.4 mM IAA. However, increased in the level of IAA in genotype 47-4 resulted to reduction in soluble sugar accumulation in fruit; IAA has no effect on soluble sugar accumulation in leaf when compared to control group (Figure 11). Similarly, in genotype LD88, increased in the levels of IAA reduced the soluble sugar accumulation in both fruit and leaf of genotype LD88, but the highest soluble sugar accumulation was seen in the fruit of the group treated with 0.4 mM IAA as compared to the control group (Figure 12).

IV. Discussion

According to (Amin *et al*., 2009), increase in the amount of proline, protein and sugars accumulation in the plants would lead to the resistance against loosing water, protect turgor, reduce the membrane damage and accelerate the growth of plants in stress conditions. The higher accumulation of proline under stress conditions was attributed to increased activities of proline biosynthesis enzymes such as ornithine aminotransferase and pyrroline-5-carboxylate reductase, as well as due to inhibition of proline degradation enzymes, proline oxidase and proline dehydrogenase (Kishor*et al*., 2005). Also, in this present study, it is obvious from the data obtained that proline concentrations in both genotypes increased as the salt stress increase, but in other way round salt stress decreased protein content and soluble sugar accumulation considerably in both genotypes. Interaction of IAA with salinity especially at 0.4 and 0.5 mM IAA alleviated the deleterious effect of salinity stress on soluble sugar accumulation as well as on protein content accumulation and thus improved protein content and soluble sugar accumulation in the okra plant, but reducing sugar concentration in the leaf of LD 88 increased with increased salinity levels. The reductions in protein content and soluble sugar concentration were more observed at severe salt stress compared to mild stress. The results obtained were in consonant with the results of Hussein *et al*., (2007); Amin *et al*., (2009); Black and Prithard, (2002); Smirnoff, (1998) and Yazdanpanah*et al*., (2011) that reported decreased in protein concentrations under abiotic conditions, which due to decreasing protein biosynthesis and decomposition of proteins as a result of reactive oxygen species generated. Also the results were in tandem with the results of Ackerson (1985) and (Srivastava*et al*. 1995) that reported accumulation of carbohydrate by the studied bioregulator, plays a key role in mediating the salinity stress, either via osmotic adjust-

ment, or by conferring some resistance to plant cells. Therefore, the present study showed that IAA treatment mitigates the negative effect of stress on okra plant via accumulation of proline, protein and soluble sugar content of okra plant and thus increased their values.

V. Conclusion

From the results and discussion, it could be inferred that pretreatment of okra seeds with indole acetic acid and salinity, induced plant and thus stimulates the salt tolerance of the okra plants via enhancing the accumulation of nontoxic metabolites (sugars, proline and protein) that ameliorate salt stress damage in plants, thereby improving the tolerance to salt stress. Thus, 0.4 and 0.5 mM of IAA serve as promising concentrations to alleviate the effects of salinity stress on okra plant, which may be due to reduction in reactive oxygen species at these concentrations.

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Notes

The authors declare no competing interest.

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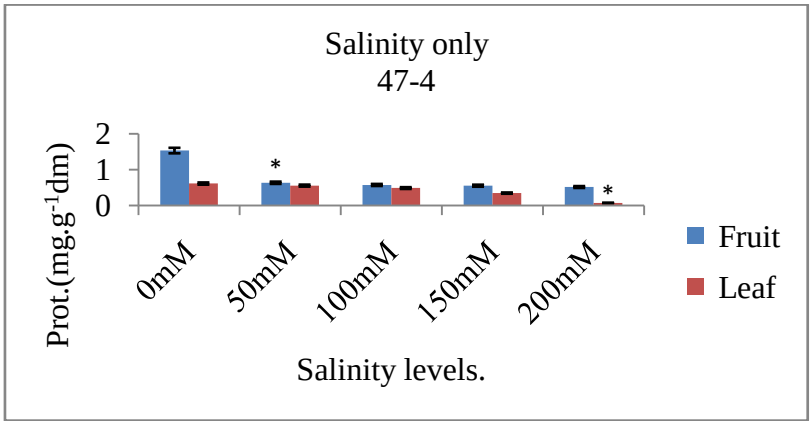
Table 1: Effect of salinity levels on proline content (Mgg^{-1} dm.) of different organs of two okra genotypes.

NaCl (mM)	Genotype 47-4		Genotype LD88	
	Leaf	Fruit	Leaf	Fruit
0	1.12±0.02	1.08±0.01	2.00±0.02	1.15±0.01
50	2.40±1.00	1.97±0.03	2.35±0.02	2.00±0.02
100	3.50±0.03	2.85±0.04	3.65±0.01	2.86±0.02
150	4.13*±1.02	4.10*±0.01	4.14*±0.05	4.37*±0.01
200	5.21*±0.01	4.79*±0.03	4.99*±0.02	5.11*±0.02

Values are the mean of three replicates mean ± S.E
* Significant different at P = 0.05 when compared with normal control

Figure Captions:
Fig. 1: Effect of salinization on soluble proteins accumulation of different organs of okra (Genotype 47-4). Vertical bars represent Standard deviation.
* Significant different at P ≤ 0.05 when compared with normal control
Fig. 2: Effect of salinization on soluble proteins accumulation of different organs of Okra (Genotype LD 88). Vertical bars represent Standard deviation.
* Significant different at P ≤ 0.05 when compared with normal control
Fig. 3: Combined effect of IAA and salinity on soluble proteins accumulation of different organs of Okra (Genotype 47-4). Vertical bars represent Standard deviation.
** Significant different at P ≤ 0.05 when compared with treated control in fruit
* Significant different at P ≤ 0.05 when compared with treated control in leaf
Fig. 4: Combined effect of IAA and salinity on soluble proteins accumulation of different organs of Okra (Genotype LD 88). Vertical bars represent Standard deviation.
** Significant different atP ≤ 0.05 when compared with treated control in fruit
* Significant different atP ≤ 0.05 when compared with treated control in leaf
Fig. 5: Effect of salinization on reducing sugar accumulation of different organs of Okra (Genotype 47-4). Vertical bars represent Standard deviation.
* Significant different atP ≤ 0.05 when compared with normal control
Fig. 6: Effect of salinization on reducing sugar accumulation of different organs of Okra (Genotype LD 88). Vertical bars represent Standard deviation.
* Significant different atP ≤ 0.05 when compared with normal control
Fig. 7: Combined effect of IAA and salinity on reducing sugar accumulation of different organs of Okra (Genotype 47-4). Vertical bars represent Standard deviation.
** Significant different atP ≤ 0.05 when compared with treated control in fruit
* Significant different at P ≤ 0.05 when compared with treated control in leaf
Fig. 8: Combined effect of IAA and salinity on reducing sugar accumulation of different organs of Okra (Genotype LD 88). Vertical bars represent Standard deviation.
**Significant different at P ≤ 0.05 when compared with treated control in fruit
* Significant different atP ≤ 0.05 when compared with treated control in leaf
Fig. 9: Effect of Indole Acetic Acid on soluble proteins accumulation of different organs of Okra (Genotype 47-4). Vertical bars represent Standard deviation.
* Significant different atP ≤ 0.05 when compared with normal control
Fig. 10: Effect of Indole Acetic Acid on soluble proteins accumulation of different organs of Okra (Genotype LD 88). Vertical bars represent Standard deviation.
* Significant different at P ≤ 0.05 when compared with normal control
Fig. 11: Effect of Indole Acetic Acid on reducing sugar accumulation of different organs of Okra (Genotype 47-4). Vertical bars represent Standard deviation.
* Significant different at P ≤ 0.05 when compared with normal control.
Fig.12: Effect of Indole Acetic Acid on reducing sugar accumulation of different organs of Okra (Genotype LD 88). Vertical bars represent Standard deviation.

* Significant different at P ≤ 0.05 when compared with normal control.



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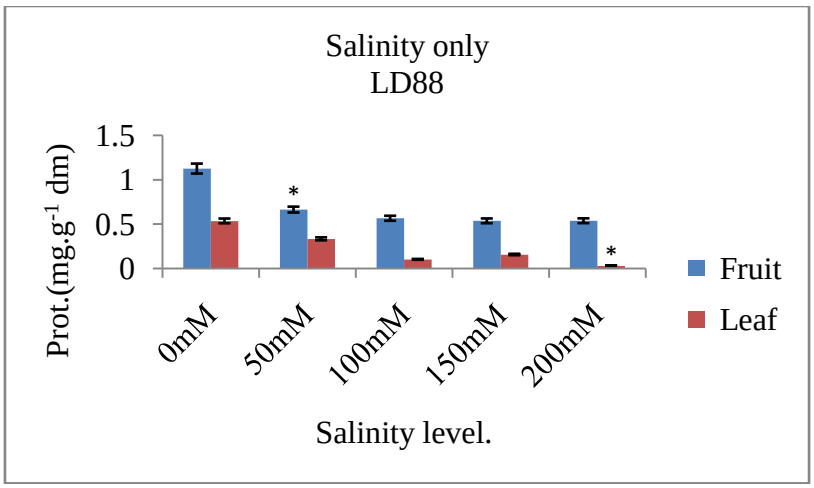

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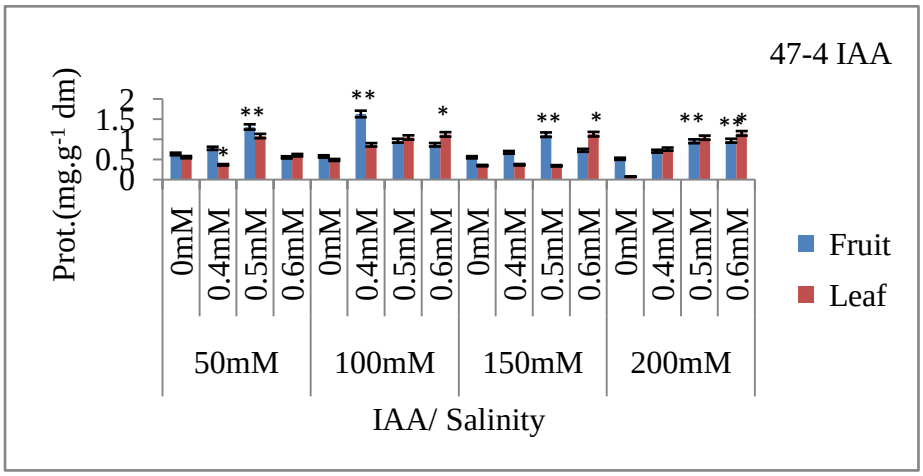

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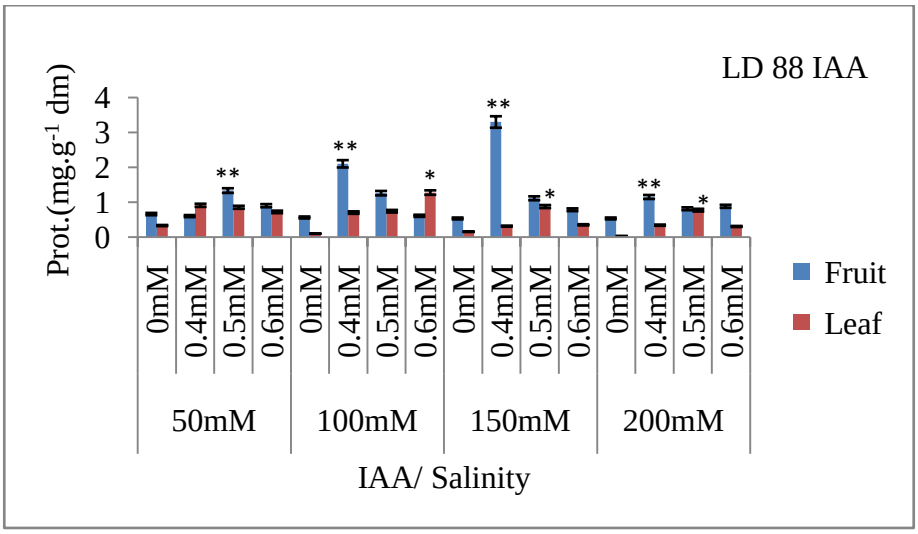

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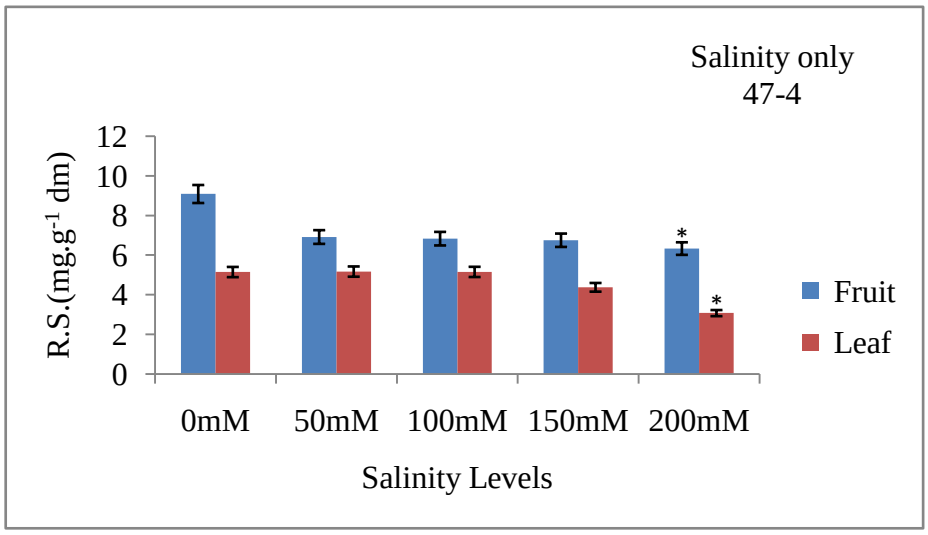

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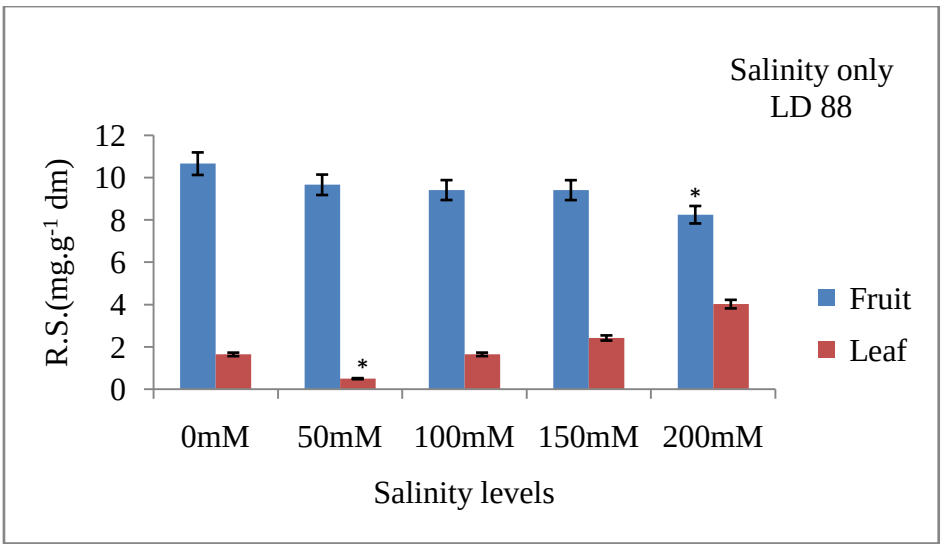

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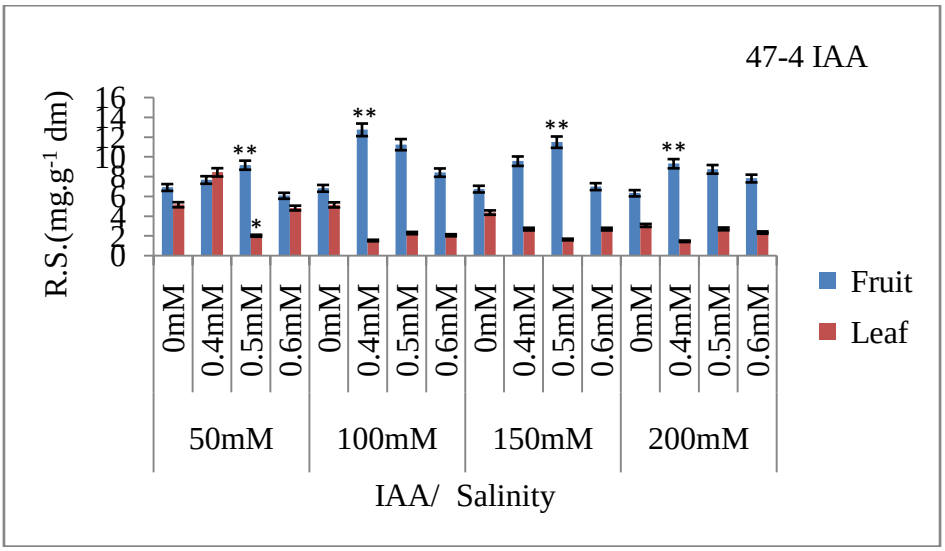

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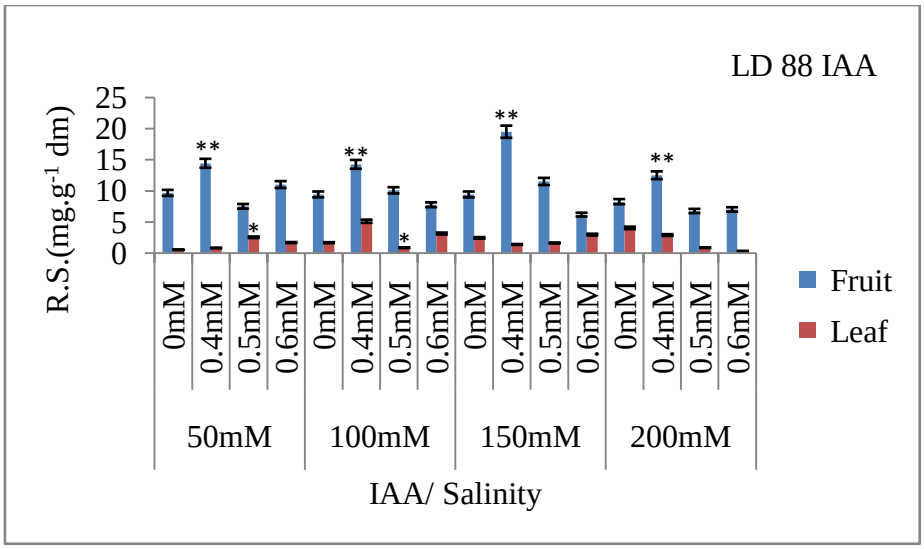

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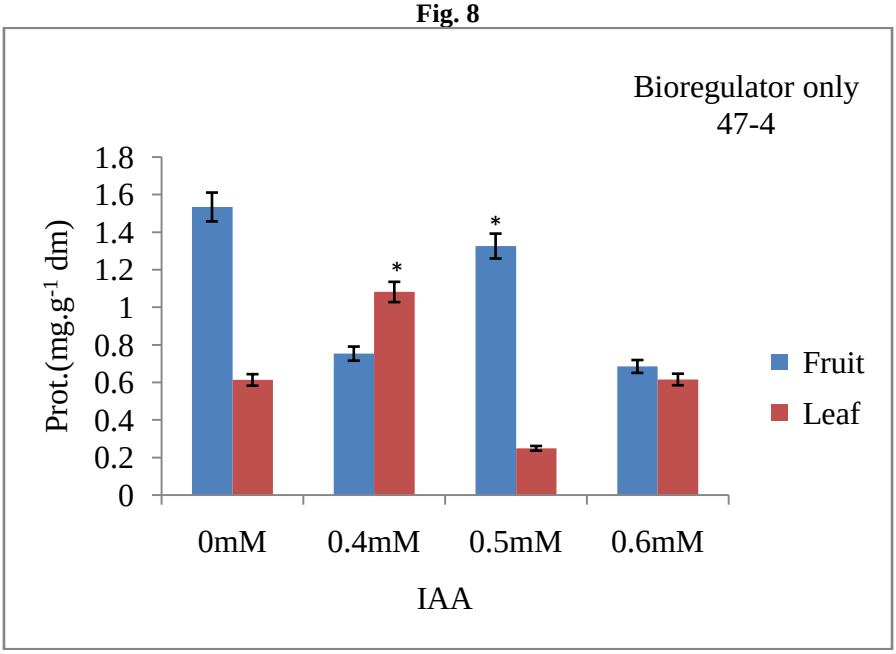

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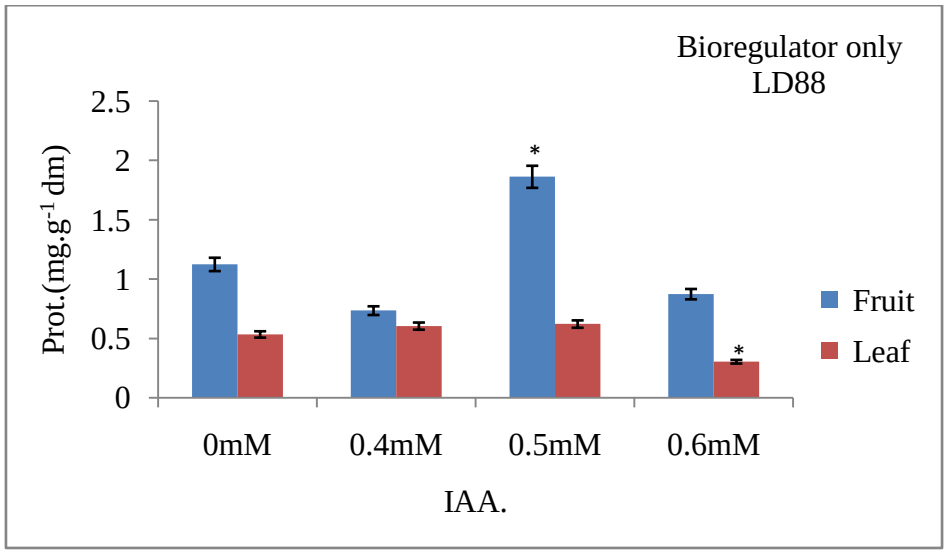

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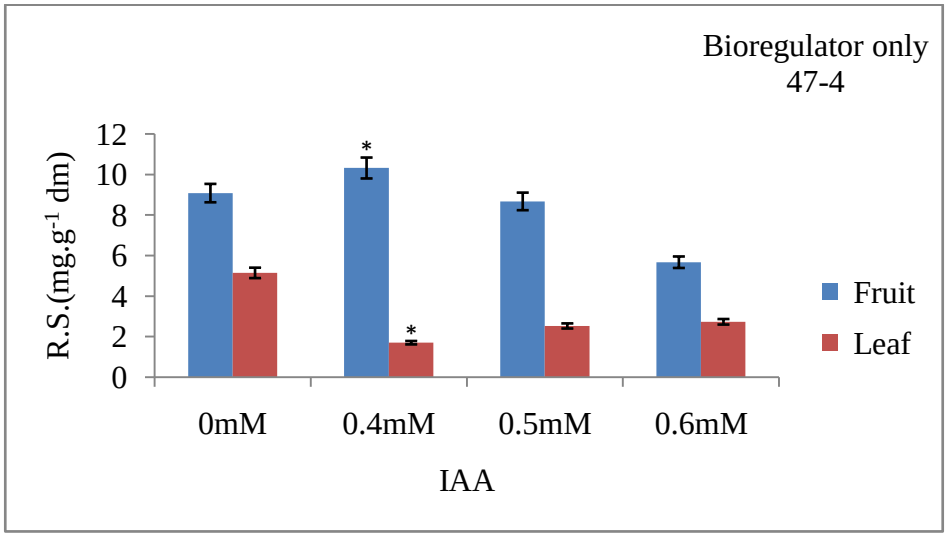

Fig. 11

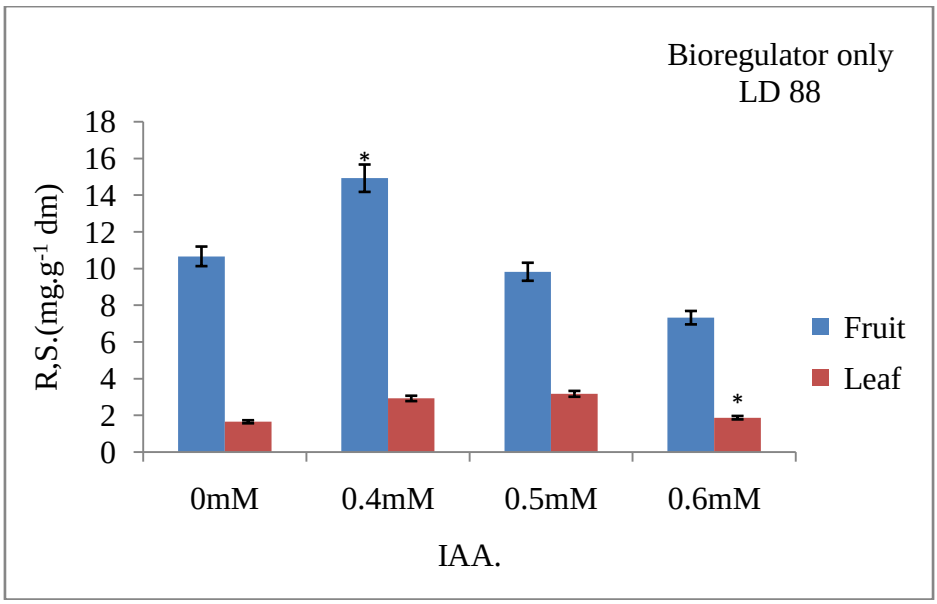

Fig. 12