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Effect of salicylic acid (SA) seeds soaking on the NaCl salt stress induced changes in soluble sugar and protein accumulation in organs of two genotypes of okra plants

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Salt stress is a major challenge in agricultural system. In this study, okra seeds of two genotypes (47-7 and LD 88) were presoaked with 10^{-2}, 10^{-4}, and 10^{-6} mM salicylic acid and control in distilled water, then the soil was treated with 0, 50, 100, 150 and 200 mM NaCl. The experiment was conducted to study the effect on osmoregulating solutes such as proline, salt stress protein (glycine betaine and proline betaine) and soluble sugars (glucose and fructose). Results showed that proline content increased with increased in the concentrations of salinity. Also, treatment with salicylic acid (SA) improved salt stress proteins accumulation in both stressed genotypes. In contrast, decreased SA concentrations improved soluble sugar accumulation in the fruit of okra genotype 47-4. But in LD88, increased in the level of SA resulted to the increased soluble sugar accumulation in the leaf. Combined effect of SA and salinity caused a greater accumulation of protein and soluble sugar in leaf and fruit of both genotypes of stressed okra, but significant increased were seen only in the groups of LD88 treated with 10^{-4} mM SA at 50 mM NaCl in leaf and 10^{-2}mM SA at 150 mM NaCl in fruit when compared with the control group. Salinity induced a marked decreased in reducing sugar accumulation of okra plant (LD88), especially at high salinity level (200 mM NaCl). Therefore, accumulations of compatible solutes such as salt stress proteins may provide plant a storage form of nitrogen that will be re-utilized later and may play a role in osmotic adjustment.

Key words: Distilled water, fruit, leaf, okra, proline, protein, salicylic acid (SA), salinity, soluble sugar.

INTRODUCTION

Abelmoschus esculentus, which is otherwise known as okra, in many English-speaking countries they know it as lady's fingers or gumbo. Okra is a flowering plant in the mallow family (Chopra et al., 1956). It is a vegetable grown widely in Nigeria for its soft fruits and young leaves. It is distributed across the Africa, Asia, Southern

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Europe, and America (Khomsug et al., 2010). In some areas, the leaves are for human consumption, the major components of okra are vitamins, mineral salts, and calcium, which is deficient in the diet of people living in developing countries (IBPGR, 1990). The plant is used for medicinal purposes and also used to treat many diseases; it induces hypoglycemic activity in normal mice (Tomoda et al., 1989). Biotic and abiotic factors such as drought, water, and salinity stress drastically affect the growth and productivity of okra plant in the tropical and sub-tropical regions of the world. Plant growth and productivity affected by salinity as one of the major environmental factors (Misra et al., 1990). It is known that up to 20% of irrigated lands in the worlds are affected by levels of salinity (Mostafazadeh-Fard et al., 2007). High concentration of salt has adverse effect on many crop species (Zörb et al., 2004), salinity affected cell enlargement as well as photosynthesis (Misra et al., 2001; Munnus et al., 2006), it has negative effect on cell division and cell growth (Maghsoudi and Maghsoudi, 2008). Exposure of plants to salt stress increase reactive oxygen species which destroy membrane lipids (Zörb et al., 2004). Plants that exposed to salt stress produced metabolite like proline, exposure of higher plants to salt stress produced proline which is free amino acid. It is highly active, and plays an important role in membrane stability, also mitigates the effects of saline on cell membrane disruption (Parviz and Satyawat, 2008). Establishment of methods to induce stress tolerance in plants is important, and still need considerable attention. Methods used to develop stress tolerant in plants included genetic engineering, traditional breeding, *in vitro* selection, and the use of growth regulators (Baninasab and Ghobadi, 2011; Senaratna et al., 2000). Salicylic acid has been known as phytohormone that plays a vital role in the controlling of plant growth and development, seed germination, fruit yield, glycolysis, flowering, and heat production in plants (Klessig and Malamy, 1994), ions uptake and transport (Harper and Balke, 1981), values of photosynthesis, stomata conductance, and transpiration (Khan et al., 2003). Salicylic acid is a growth regulator with phenolic nature (Sakhabutdinova et al., 2003), it acts as non-enzymatic antioxidant, also plays a vital role in regulating some plant physiological processes (Noreen et al., 2009), such as stimulating adventitious organ, development, herbicidal effect and providing resistant to environmental stress (Hussein et al., 2007).

To withstand different environmental factors, plants alter their metabolic pathways to adjust to changes in environments (Rathinasabapathi, 2000), compatible solutes made up of a wide range of organic compounds, such as: Simple sugars (fructose and glucose), sugar alcohols (glycerol and methylated inositols), complex sugars (trehalose, raffinose and fructans), polyols, quaternary ammonium compounds (proline, glycine betaine, alpha-alanine betaine, proline betaine) and tertiary sulfonium compounds that are hydrophilic, they can replace water at the surface of proteins, complex protein structures and membranes which explains their action as osmoprotectants and as low molecular weight chaperones (Hasegawa et al., 2000; Nuccio et al., 1999). The metabolic pathways such as proline, glycine betaine, polyols, antioxidant components are responsible to keep the plant survive under stress conditions. Proline is the most common organic compatible solute in the cytoplasm and organelles to keep stability of osmotic pressure of ions in the vacuole, high level of proline may improve the osmotic adaptation and protect the plants against the salt or drought induced injuries. Under salt stress, proline is significantly accumulated and performs the positive role in the adaptation of cells to salt and water stress (Kaviani, 2008). Proline plays a major 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 salicylic acid on negative effects of saline, as well as on accumulations of compatible solutes, and also to determine the best concentration of salicylic acid that accumulates more of these metabolic constituents in stressed okra plant.

MATERIALS AND METHODS

Plant growth conditions

The seeds of okra plant used for this work were obtained from genetic resource laboratory of National Horticultural Research Institute (NIHORT), Ibadan, Oyo state, Nigeria. Seeds were sterilized with 1% sodium hypochlorite for 15 min and washed twice with double distilled water. The seeds were then soaked with 10^{-2}, 10^{-4} and 10^{-6} mM of salicylic acid and distilled water (control) for 12 h and then placed to germinate in polyethylene bags containing 10 kg of soil with pH 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. Seeds were grown in the soil that contains no saline (control (0 mM)) and other seeds were grown under salinity levels of 50, 100, 150, and 200 mM. Saline solutions were added to the soil until field capacity was reached.

The experiment was done in a screen house at National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. There were 60 polyethylene bags for each genotype (3 treatments x 5 levels x 3 replicates), and they were moisture with normal tap water on weekly basis to attain soil water field capacity for the period of eight weeks. After which metabolic constituents were determined on dried leaf and fruit of the two genotypes.

Proline determination

Proline levels in leaf and in the fruit were determined according to the method of Bates et al. (1973) with slight modification. Five-hundred milligrams of the dried leaf and fruit samples were dissolved in 10 mL of 3% (v/v) aqueous sulfosalicylic acid. The mixture was filtered using Whatman no 41 filter paper. After which the filtrate was acidified with glacial acetic acid and ninhydrin (1 mL each) and then heated in water bath at 100°C for 1 h. The mixture

Table 1. Effect of salinity levels on proline content (mgg^{-1}dry matter) of different organs of two okra genotypes.

NaCl (mM)	Genotype 47-4		Genotype LD88	
	Leaf	Fruit	Leaf	Fruit
Control (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 group.

was extracted with 5 mL of toluene and the upper phase was decanted into a glass cuvette, and then the absorbance was taken at 520 nm.

Soluble protein determination

Proteins concentrations were analyzed according to 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 at 660 nm by spectrophotometer machine (Pharmaspec UV-1700 model). The amount of soluble protein was calculated from bovine serum albumin standard curve.

Soluble sugar determination

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

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.

RESULTS AND DISCUSSION

As shown in Table 1, in the two genotypes of okra, as the salinity level increased, proline accumulation also increased in leaf and in the fruit when compared with control group. This has also been reported by Amin et al. (2009) that increased in the amount of proline, protein and sugars 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. Under stress conditions, the higher proline concentration increases the activities of proline biosynthesis enzymes such as; ornithine aminotransferase and pyrroline-5-carboxylate reductase, as well as due to inhibition of proline degradation enzymes like proline oxidase and proline dehydrogenase (Kishor et al., 2005). Figure 1 showed decreased soluble protein accumulation in the leaf as concentration of salicylic acid decreased, but in fruit, SA has little or no effect on protein accumulation but nevertheless, the group treated with 10^{-4} mM SA showed highest accumulation of protein when compared with control group of genotype 47-4. But interaction of SA and salinity significantly affected protein accumulation in leaf and fruit of okra plant (genotype 47-4) in the group treated with 200 mM NaCl, whereas significant accumulation of soluble protein were seen at mild and moderate levels of salinity. On the contrary to protein accumulation in 47-4 genotype, in Figure 2, there were appreciable increased reducing sugar accumulation in the groups treated with 10^{-2} and 10^{-6}mM of SA, and the highest accumulation of reducing sugar level in the fruit of the okra genotype 47-4 was recorded in group treated with 10^{-6} mM of SA when compared with control group, but in leaf no significant effect was recorded. Combined effect of SA and salinity showed increased reducing sugar accumulation in the fruit of okra plant (genotype 47-4) at higher salinity levels (100 and 150 mM NaCl at 10^{-2} and 10^{-4} mM SA) respectively, as compared to treated control group.

Similarly, in LD88, the leaf of okra showed decreased protein accumulation level as the concentration of SA decreased, fruit exhibited highest protein accumulation in group treated with 10^{-4}mM of SA as compared to control group. Interaction of SA and salinity significantly affected the protein accumulation in both leaf and fruit of the stressed okra, but significant increase of protein accumulation were seen only in the groups (LD88) treated with 10^{-4} mM SA at 50 mM NaCl in leaf and 10^{-2} mM SA at 150 mM NaCl in fruit when compared with the treated control group (Figure 3). In LD88, reducing sugar accumulation decreased in the leaf as salicylic acid decreased, but fruit exhibited highest reducing sugar accumulation in the group treated with 10^{-4}mM SA when compared with control group, salinity induced a marked decreased reducing sugar accumulation in okra leaf (LD88) especially at salinity levels (50 and 100 mM NaCl). Fruit showed appreciable accumulation of reducing sugar in the group treated with 10^{-6} mM of SA at

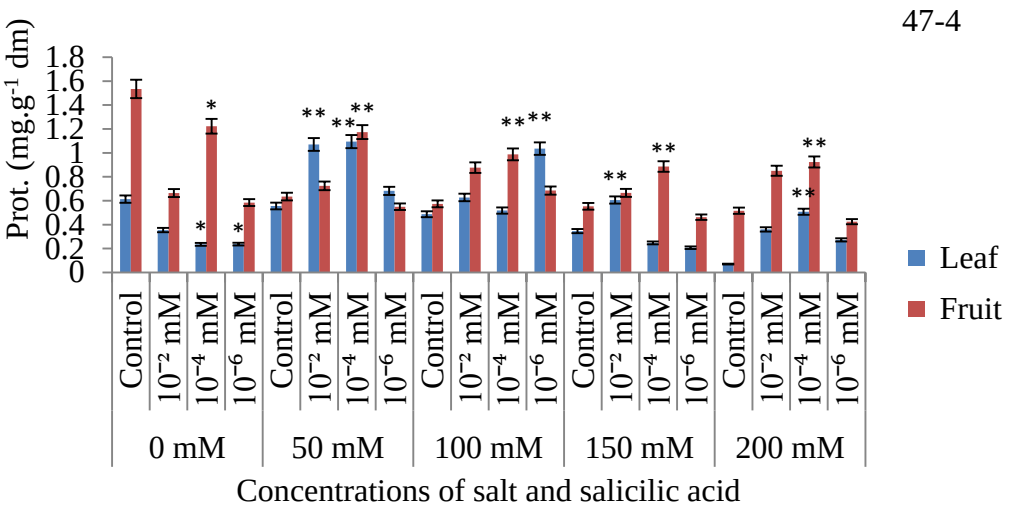

Figure 1. Effect of salicylic acid and salinity on soluble proteins accumulation in different organs of okra (Genotype 47-4). Vertical bars represent standard deviation. ** Significant different at P ≤ 0.05 when compared with treated control group; * Significant different at P ≤ 0.05 when compared with normal control group; 0, 50, 100, 150 and 200 mM are contractions of sodium chloride (salt);10^{-2}, 10^{-4} and 10^{-6} mM are concentrations of salicylic acid (SA).

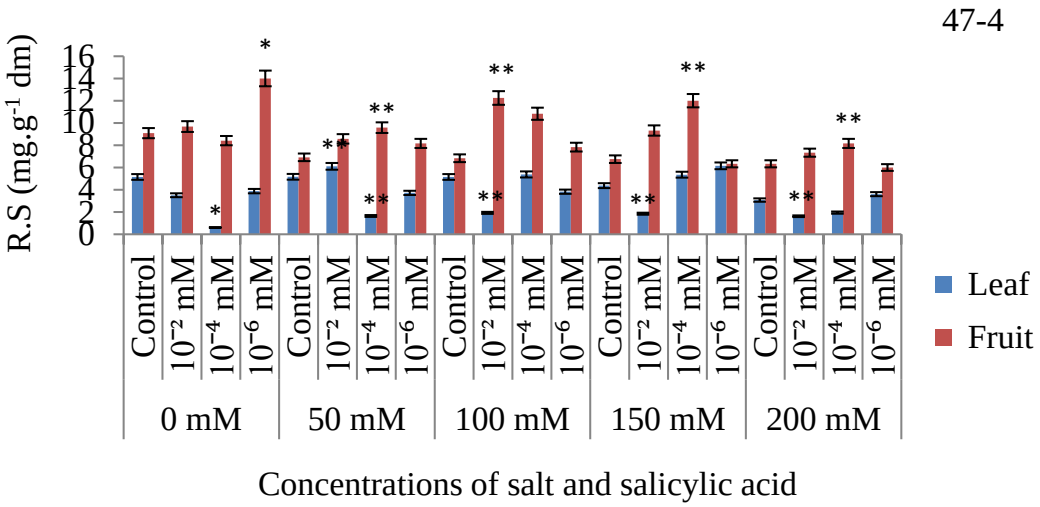

Figure 2. Effect of salicylic acid and salinity on reducing sugar accumulation in different organs of okra (Genotype 47-4). Vertical bars represent Standard deviation. ** Significant different at P ≤ 0.05 when compared with treated control group; * Significant different at P ≤ 0.05 when compared with normal control group; 0, 50, 100, 150 and 200 mM are contractions of sodium chloride; 10^{-2}, 10^{-4} and 10^{-6} mM are concentrations of salicylic acid.

50 mM NaCl, but interaction of SA and salinity in leaf showed little or no effect on reducing sugar accumulation as compared to treated control group (Figure 4).

Between the two organs of stressed okra plant, fruit accumulates more soluble sugar and soluble protein than the leaf in the two genotypes. The results were in tandem with those of Richardson and McCree (1985). They observed that increased in the concentration of solutes in plant tissues will determine its tolerance to stress conditions. Therefore, the sensitivity of both genotypes was associated with lowering soluble protein in both leaf and fruit at high salinity level, which was more severe in LD88 genotype. This result was in accordance with the findings of Marcelis and Van Hooijdank (1999), Misra et al. (1995) and Das et al. (1990). From the results obtained, pretreatment with salicylic acid or salicylic acid

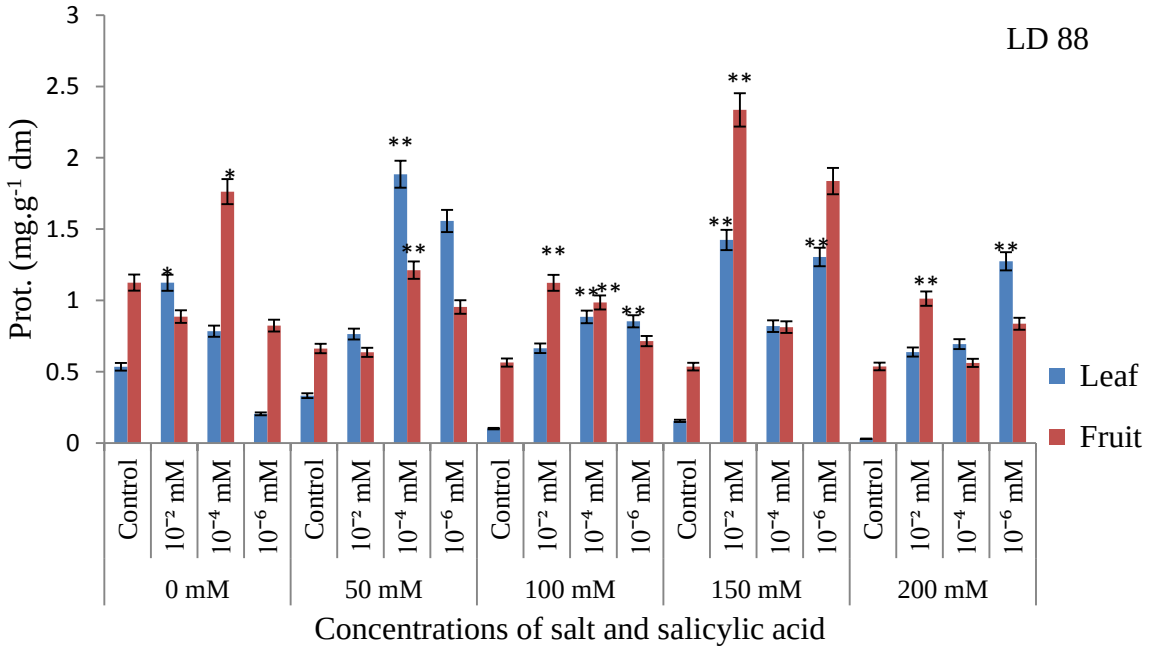

Figure 3. Effect of salicylic acid and salinity on soluble proteins accumulation in different organs of okra (Genotype LD 88). Vertical bars represent standard deviation. ** Significant different at P ≤ 0.05 when compared with treated control group; *Significant different at P ≤ 0.05 when compared with normal control group; 0, 50, 100, 150 and 200 mM are contractions of sodium chloride; 10^{-2}, 10^{-4} and 10^{-6} mM are concentrations of salicylic acid.

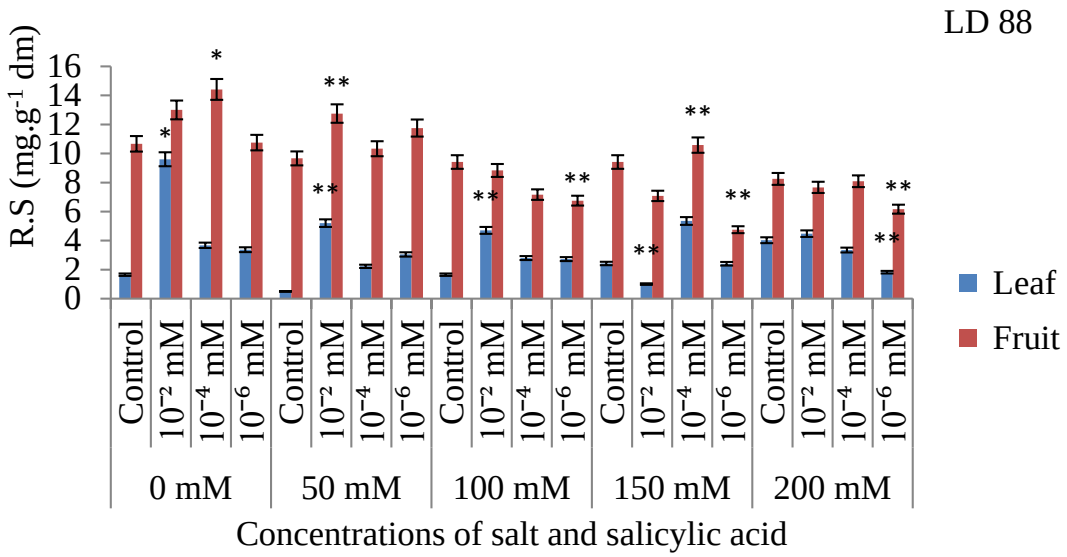

Figure 4. Effect of salicylic acid and salinity on reducing sugar accumulation of different organs of Okra (Genotype LD 88). Vertical bars represent standard deviation. **Significant different at P ≤ 0.05 when compared with treated control group; * Significant different at P ≤ 0.05 when compared with normal control group; 0, 50, 100, 150 and 200 mM are contractions of sodium chloride; 10^{-2}, 10^{-4} and 10^{-6} mM are concentrations of salicylic acid.

combined with salinity improved okra plant tolerance to salinity stressed via increasing the accumulation of non-toxic metabolites (soluble sugars, soluble proteins and proline), which reflected more in the fruit than leaf of okra plant. Therefore, exogenous application of growth regulator especially salicylic acid could be applied to

improve okra plant salt stress tolerance at 10^{-2} and 10^{-4}mM concentrations.

Conflict of Interests

The authors have not declared any conflict of interests.

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