Response of salt-stressed Dolichos lablab L. seedlings to supplementary salicylic acid and its derivatives

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Abstract

Plants exposed to salinity stress undergo adverse changes in their key physiological and biochemical parameters. The work reported in this paper assessed the effect of exogenous application of salicylic acid, acetyl salicylic acid (aspirin) and methyl salicylate (5mM each) on proteins, soluble sugars and chlorophyll content in salt-stressed (50mM NaCl) *Dolichos lablab* leaves. Salinity significantly diminished proteins and chlorophylls and increased soluble sugars relative to controls. Exogenous application of salicylic acid and its derivatives as foliar spray substantially enhanced and alleviated the deleterious effects of salinity on the studied parameters.

1. Introduction

Salinity is the accumulation of salt in soil and water. High soil levels can adversely affect plant growth, soil structure, water quality and infrastructure. Salinization of soils is one of the major factors limiting crop production particularly in arid and semi-arid regions of the world (Ahmed, 2009). In the world about 830 million hectares are affected by salt (El Naim et al., 2012). Sudan had about 4.8 million hectares are salt affected to various degrees (FAO, 2000). Salt stress is known to perturb a multitude of physiological processes (Noreen and Ashraf, 2008). It exerts its undesirable effects through osmotic inhibition and ion toxicity (Munns 2008).

Damages generated by salt stress can be alleviated or reduced either genetically (by breeding of salt tolerant plants) or biochemically by application of chemical signaling molecules such as plant growth regulators (Khairy and Roh, 2016). It is reported that application of signaling molecules like salicylic acid and its derivatives can enhance salt tolerance in plants by inducing protective effects of plants under salinity stress (Raskin, 1992).

Many plant regulators play an important role in regulating the growth under stressful environment. Salicylic acid is a common plant-produced phenolic compound, which can function as growth regulator (Arberg, 1981). In addition, salicylic acid could be induced in the category of phytohormones (Raskin, 1992). Exogenous application of salicylic acid may influence a range of processes in plants, including anti-oxidative enzyme activity (Parida and Das, 2005), seed germination Borsani et al., 2007), ion uptake and transport (Harper and Balke, 1981), disease resistance (Park et al., 2009) photosynthesis (Khan et al., 2003) and stomatal closure (Larque – Saavedra, 1979).

Accordingly, the present study was designed to verify the effect of exogenous application of salicylic acid and its derivatives, on mitigating the depressive effects of salinity and improving key physiological and biochemical parameters in salt-stressed seedlings of *Dolichos lablab* L.

2. Materials and Methods

2.1 Plant culture:

Seeds of *Dolichos lablab* (louba afin) cultivar SHDLO5 obtained from the Faculty of Agriculture, University of Khartoum were used in this study. The seeds were surface sterilized with 50% alcohol to avoid fungal contamination, and then rinsed several times with distilled water. The seeds were germinated in pots containing sand and clay (1:1). Seedlings of comparable size and age were used in this study.

The parameters were estimated after 5 days of foliary spraying salicylic acid (SA), acetyl salicylic acid (ASA), and methy salicylate (MS, 5mM each) to salt-stressed seedlings grown under 50mM NaCl.

2.2 Protein assay (Biuret method):

The method used was that described by Plummer (1978). The Biuret reagent was prepared by dissolving 3 g of Cu SO_4. H_2O and 9g of sodium potassium tartarate in 500ml of 0.2N NaOH. Five g of Kl were added, and the reagent was made to l liter with 0.2N NaOH.

To 0.2 grams of dry tissue (The plant materials were oven dried at 70°C for at least 2 days), 10ml distilled water were added and then centrifuged at 3500rpm for 5 minutes. The supernatant was used for analysis. Three ml of Biuret reagent were added to 2ml of protein solution and the mixture was warmed at 37°C for 10 minutes, cooled and absorbance was read at 540nm in a spectrophotometer. Content of proteins was derived from a standard curve prepared by egg albumin.

2.3 Soluble sugars assay:

Soluble sugars were extracted by grinding 0.1 gram of fresh plant material in pestle and

mortar with 80% ethanol. The extract was filtered through Whatman filter paper No. 1.

Two further extractions from the residue were done. The combined filtrates were heated in a water-bath to evaporate the ethanol. The remaining residue was dissolved by distilled water and made to 10 ml.

Total soluble sugars were assayed using the anthrone method (Halhol and Kleinberger 1972). Six ml of anthrone reagent (0.15% in conc. H_2SO_4) were added to 0.4 ml of distilled water and 0.1 ml of aliquot extract in a test tube, which was placed in a boiling water-bath for exactly 6 minutes. Afterwards, the test tube was transferred to cold water to stop the reaction. The tube was shaken thoroughly before reading the absorbance at 630 nm in spectrophotometer. Concentrations were derived from a standard curve.

2.4 Estimation of chlorophyll a and b:

The method used was that described by Strain and Svec, (1966).

One gram of fresh leaves is ground with 40ml of acetone solution 80% (v/v) in a clean mortar.

The green liquid is carefully transferred to Buchner funnel with Whatman filter paper No. 1. The residue is further extracted twice with acetone solution, and the filtrates were collected and made with acetone solution to 100ml.

The absorbance of the green solution is read at 645nm and 663nm- against a solvent blank.

2.5 Calculation:

The chlorophyll content is calculated as follows:

Chlorophyll a mg/ml = 11.46 x A663 - 2.16 x A645

Chlorophyll b mg/ml = 20.97 x A645 - 3.9 x A663

3. Results and Discussion

The protein content decreased in leaves of salt-stressed louba afin by 29% relative to controls (Fig. 1). Results obtained are consistent with Alamgir and Ali (1999) who showed that salinity diminished the protein content in rice. Wang and Nil (2000) have observed reduction of protein concentration in *Amaranthus tricolor* leaves during salt stress. In addition to the toxicity caused by ions, salinity and osmotic stress cause an imbalance of nutrients in plants which affects the nutrients involved in protein synthesis. El-Shintinawy and El-Shourbagy (2001) have recorded that amino acids such as cysteine, arginine and methionine which constitute about 55% of total free amino acids decrease when exposed to salinity stress.

It is noteworthy that foliar application of salicylic acid and its derivatives alleviated the depressive effects of salinity on the protein content of louba afin leaves (Fig. 1), but values still remained below controls.

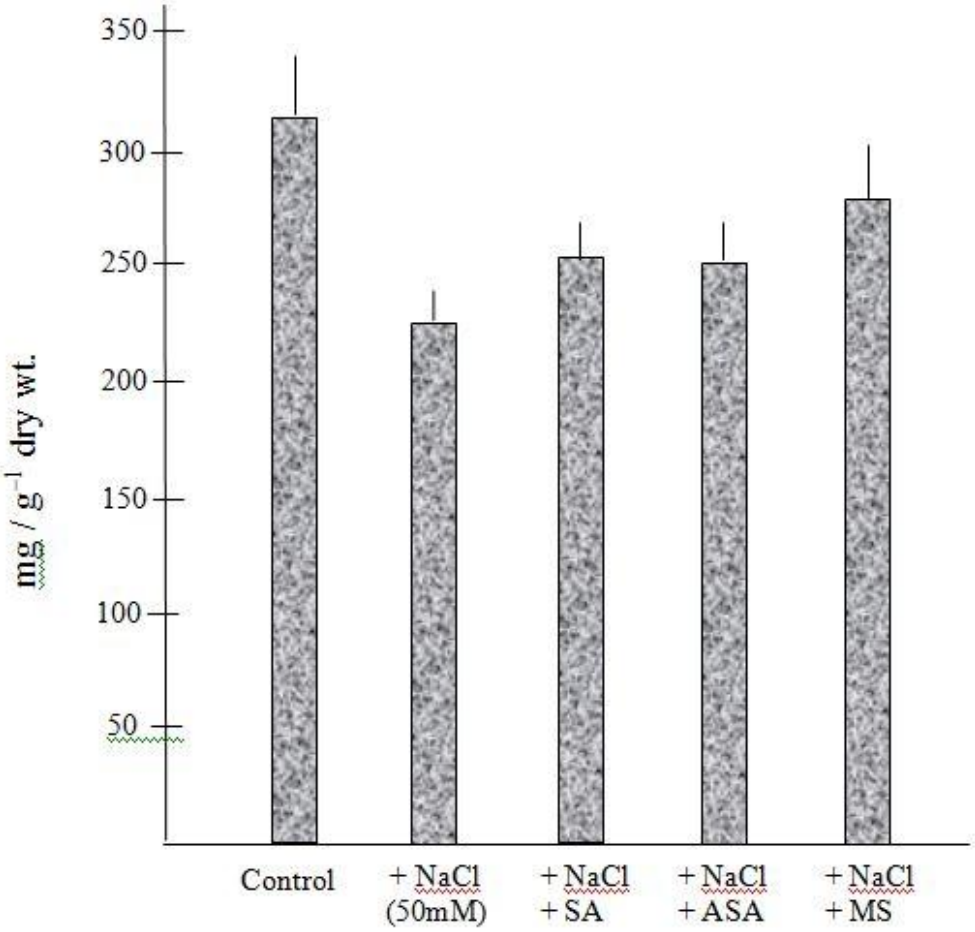

Fig. 1: Effect of salinity and salicylates supplements on the protein content of louba afin leaves. Error bars represent ± standard deviations of three replicates.

Total soluble sugars increased in salt-stressed leaves of louba afin by 23% (Fig. 2). Foliar supplements of salicylic acid and its derivatives further increased the soluble sugars by varying degrees.

Increase of total soluble sugars in salt-stressed plants is probably for adjusting osmotic potential and better water uptake. This mechanism help plants to avoid tissue death and enable them to continue their growth under saline conditions.

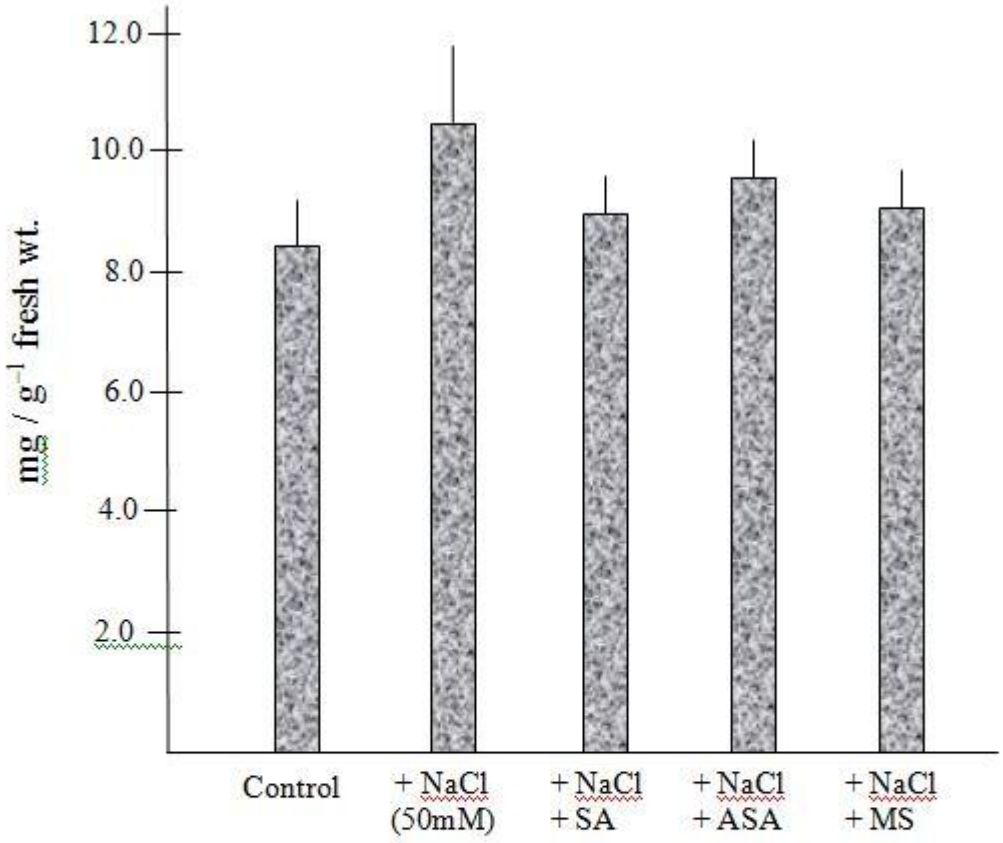

Fig. 2: Effect of salinity and salicylates supplements on the total soluble sugars in the leaves of salt-stressed louba afin seedlings. Error bars represent ± standard deviations of three replicates.

Carbohydrates such as sugars (glucose, fructose, sucrose, fructans) and starch accumulate under salt stress (Parida and Das, 2005). Their major functions are osmo-protection, osmotic adjustment, carbon storage and radical scavenging. Salt stress increases reducing sugars in a number of plants (Singh et al., 2000). The contents of reducing and non-

reducing sugars and the activity of sucrose phosphate synthetase increase under saline conditions (Dubey and Singh, 1999). Khavarinejad and Mostofi (1998) have shown that in leaves of tomato the contents of soluble sugars and total saccharides are significantly increased under saline conditions. Similar results were reported by (Parida and Das, 2005).

To satisfy the ionic balance in the vacuoles, cytoplasm accumulates low-molecular-mass compounds termed compatible solutes because they do not interfere with normal biochemical reactions (Zhifang and Loescher, 2003). With accumulation of these compounds continued water influx to cells is guaranteed. These compatible solutes include soluble sugars. To cope with salt stress plants respond with physiological and biochemical changes that aim at the retension of water despite high external osmoticum.

Results presented in (Figs. 3) demonstrate the influence of salinity and foliar application of salicylates on chlorophylls a and b. It is evident that salt-stress reduced the two chlorophylls and foliar spray of salicylates completely nullified the negative effects of salinity on the two chlorophylls, and even enhanced the concentration of the two chlorophylls above control concentrations. It is noteworthy that chlorophyll a outclassed chlorophyll b. Results obtained are consistent with Amira and Abdul Qados (2011) and Iqbal et al., (2006).

Reduction of chlorophylls observed in this study might be attributed to the toxic action of NaCl on the biosynthesis of pigments, increasing their degradation and/or maintaining damage of the chloroplast thylakoid (Rao and Rao, 1981).

Electron microscopy shows that the thylakoidal structure of the chloroplasts becomes disorganized, the number and size of plastoglobuli increases, and their starch content decreases in plants treated with NaCl (Hernandez et al., 1995).

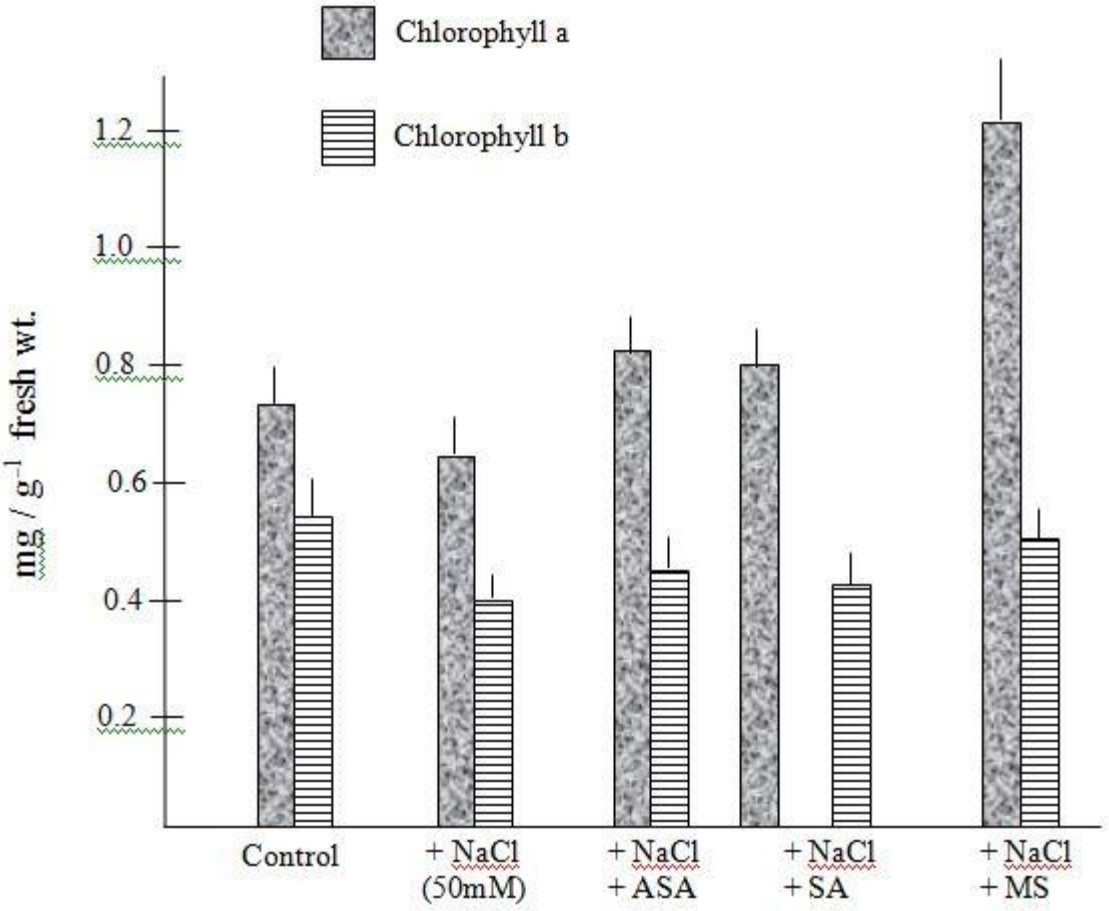

Fig. 3: Effect of foliar application of salicylates (SA, ASA, MS) on the chlorophylls content (a and b) in the leaves of salt-stressed louba afin seedlings. Error bars represent ± standard deviations of three replicates.

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