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Research Article

PROTECTIVE EFFECT OF SALICYLIC ACID ON ALUMINIUM INDUCED STRESS IN *SORGHUM BICOLOR* VARIETIES

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ABSTRACT
Sorghum bicolor is an important food crop belonging to poaceae family of monocotyledonous flowering plants. In our present study we investigated that the protective effect of Salicylic acid (250 µM) on plant growth parameters, chlorophyll and Relative water content under $AlCl_3$ (250 and 500 µM) induced toxicity at 4.5 pH in *Sorghum bicolor* varieties such as SPV1359 (Al resistance) and NTJ2(Al sensitive). The plant growth parameters such as seed germination rate shoot, root lengths, fresh and dry biomass, chlorophyll content and relative water content were decreased with their respective Aluminium (Al) concentrations. Salicylic acid treatments reduced toxicity against Al treatment and minor changes were observed in seed germination rate, shoot, root lengths, Fresh, Dry weights, chlorophyll content and relative water content. Our results clearly showed that alleviating effect of Salicylic acid against Al induced toxicity in both Al resistance (SPV1359) and Al sensitive (NTJ2) *sorghum bicolor* varieties.

Key Words: *Sorghum Bicolor, Al Toxicity, Biomass, Relative Water Content*

INTRODUCTION
The earth is gradually polluting with the releasing of several heavy metals from industries (Forstner,1995). These metals are accumulated and showed their toxic effect in plant system (Sharma *et al*., 2004, Lokeshwari and Chandrappa, 2006). Al was distributed in water, soil and air but most of the Al was stored as aluminosilicate soil minerals in the earth and toxic to plants only in acidic solid (Kochian 1995, May and Nordstro, 1991). Aluminium (Al) toxicity promotes oxidative damage in plants. The non toxic forms of Al silicates and oxides solubulized and converted in to toxic forms Al^{3+} ions at low P^H (Hoekenga *et al*. 2003). Al inhibits the root growth within few hours even at low concentration of Al and it the major phyto toxic symptom of Al (Ownby and Popham, 1989; Ryan *et al*., 1993; Chang *et al*., 1999 and Kochian, 1995). The root growth was inhibited due to the association of 95% of Al with the cell wall of the root apex and appreciable quantities of Al entered into root symplast according to (Delhaize and Ryan 1995; Tice *et al*., 1992 and Lazof *et al*., 1994). In this paper we emphasized on protective effect of salicylic acid on aluminium induced stress in *sorghum bicolor* varieties.

MATERIALS AND METHODS
Plants and Treatments
Seeds of Sorghum bicolor SPV1359 (Al resistance) and NTJ2 (Al sensitive) were obtained from ICRISAT, Hyderabad, India. The seeds were fully rinsed with distilled water and then germinated at 25 °C with 14-h photoperiod. The germinated equal length of seedlings were transferred to a net tray floated on a container filled with of 0.5 mM $CaCl_2$ solution at pH 4.5 containing 0, 250, 500 µM $AlCl_3$, 250 µM Al + 250 µM SA and 500 µM Al + 250 µM SA for 72hr. The solution was renewed daily. The root, shoot lengths and Fresh and dry weights was measured after treatment period. All the experiments were conducted in the growth chamber with a 14-h photoperiod, day/night temperature of 26/23°C, irradiance of 150 µmol (photon) m^{-2} s^{-1} and a relative humidity of 70 %.

Seed Germination Test

Triplicates of selected 25 seeds of *Sorghum bicolor* were spread on two layers of filter paper in 11- cm Petri dishes. These plates were treated with different concentrations Al Cl_3 solution (250, 500 µM $AlCl_3$, 250 µM Al + 250 µM SA and 500 µM Al + 250 µM SA) and Petri dishes were incubated in a grown chamber at 25ºC and 12 h photoperiod. The seeds were considered as germination when radical emerged 1mm and germination was calculated in Percentage.

Measurement of Fresh and Dry weights of Fresh weights (F.W) of Al treated were measured immediately after harvesting. Dry weights (D.W) were measured after oven-drying of the seedling sample or 48hr at 105^0C.

Relative Water Content (RWC)

Relative water content (RW) was determined according to (Smart and Binghum, 1974) and calculated as: RWC = (fresh mass – dry mass) / (fresh saturated mass – dry mass) X 100. Fully saturated mass (f.s.m.) was determined after floating of shoot sample on distilled water for 4hr at 20^0C in darkness.

Pigments Analysis

Photosynthetic pigments Chlorophyll 'a' and chlorophyll 'b' and carotenoids were measured according to (Sims and Gamon 2002). Chlorophylls and carotenoids were extracted with acetone / 50Mm Tris buffer at PH 7.8 (80:20) (V/V). After homogenization and centrifugation, supernatant was used and absorbance read at 663nm, 537nm and 470nm for chlorophylls and carotenoids determination.

Chlorophyll –'a = 14.85 A664.9 – 5.14 A648.2

Chlorophyll – b = 25.48 A648.2 – 7.36A664.9

RESULTS

Plant growth parameters (Seed germination rate, Fresh and dry weights, Root and shoot lengths.)

Effect of various concentrations of Al and SA on Seed germination rate of Sorghum varieties. Seed Germination rate was scored as germinated when the breakage of seed coat was visible at after 72hrs imbibitions. Data are means ± SD (n = 4).

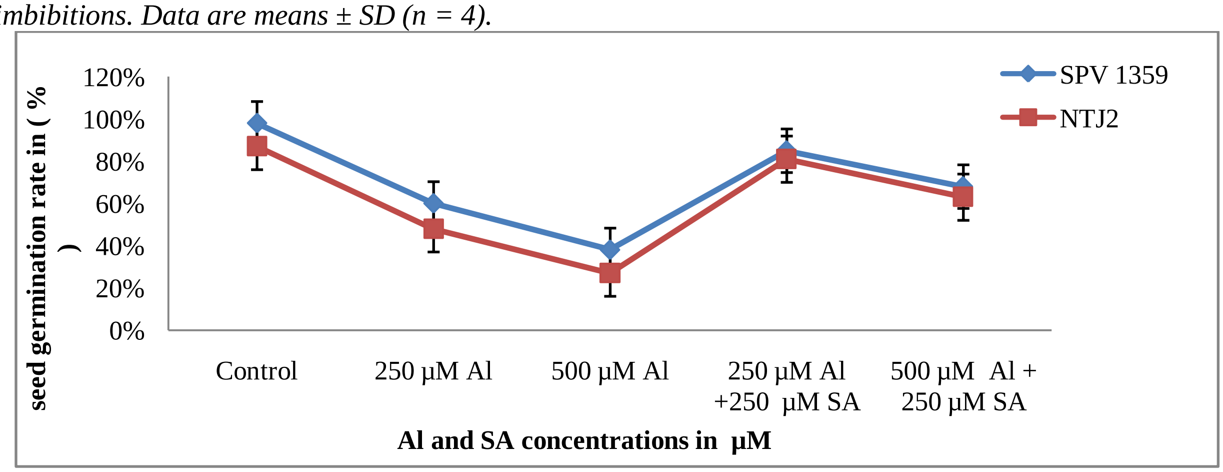

Figure 1: Effect of Al and SA on seed germination rate in SPV1359 (Al resistance) and NTJ2 (Al sensitive) Sorghum varieties

The elevated levels of Al concentration significantly reduced the seed germination rate in both SPV1359 (Al resistance) and NTJ2 (Al sensitive) sorghum varieties (Fig-1). With the increasing concentration of Al 0, 250 and 500µM the seed germination rate was decreased (98.56 ± 0.04 %, 60.25± .0.09%, 38.45±0.06 %.) in Al resistance and (87.45± 0.05%, 48.75±0.12%, 27.21±0.04% in Al sensitive (NTJ2). Seed germination rate was recovered (85.41±0.07%, 68.84±0.09%) in Al resistance (SPV1359) and (81.85±0.02%, 63.15±0.84%) in Al sensitive (NTJ2) after treatment with 250 µM SA against 250 µM and 500 µM Al respectively (Fig.1).

Effect of various concentrations of Al and SA on Root and shoot lengths of Sorghum varieties. Root and shoot lengths were measured by using scale. Data are means ± SD (n = 4).

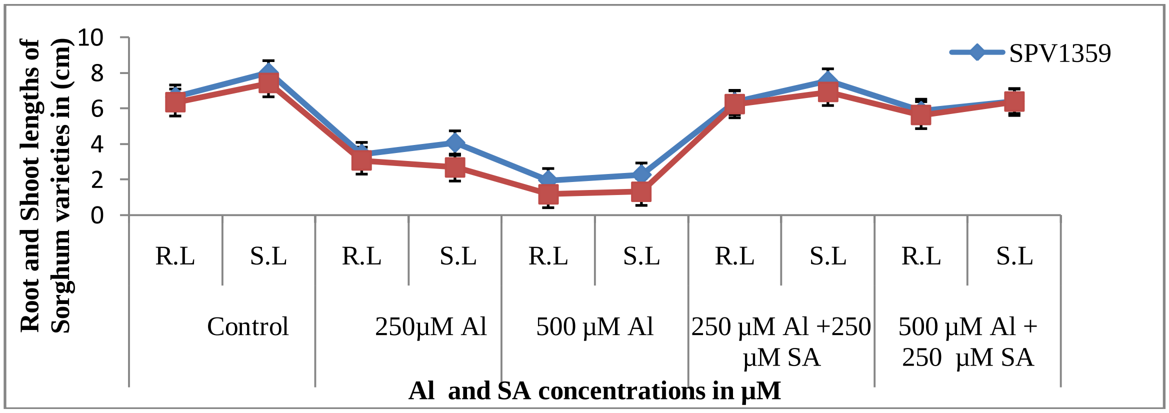

Figure 2: Effect of Al on Shoot and Root lengths of SPV1359 (Al resistance) and NTJ2 (Al sensitive) Sorghum varieties

The root and shoot lengths were also decreased after Al treatment in both sorghum varieties along with the increasing concentration of Al. the significant reduction of shoot (1.95±0.21, 2.26±0.17) and root lengths (1.19±0.25, 1.32±0.16) was observed at 500µM Al concentration. The root (5.85±0.12, 6.42±0.05) and shoot lengths (5.64±0.15, 6.38±0.06) were increased significantly after treatment with SA (250 µl) against Al (Fig.2).

Effect of various concentration of A and SA on Fresh and Dry weights of Sorghum varieties after three days treatment. Fresh and dry weights were measured in mg. Data are means ± SD (n = 4).

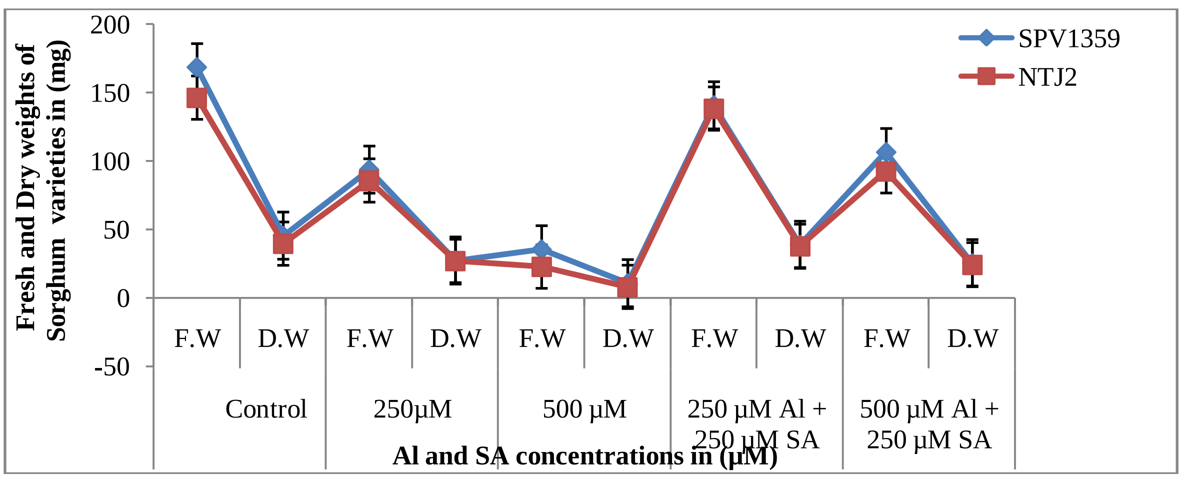

Figure 3: Effect of Al and SA on Fresh and Dry weights of Al treated SPV1359 (Al resistance) and NTJ2 (Al sensitive) Sorghum varieties

In both Al resistance and sensitive sorghum varieties Fresh and dry weights were significantly reduced with the increasing concentration of Al (250 and 500 µl). At higher concentration of Al (500 µl) the fresh weights (35.36±0.04, 10.56±0.09 mg) and dry weights (22.65±0.03, 7.86±0.02mg) were reduced significantly. SA treatment (250 µl) with (500 µl Al) was increased the fresh (106.45±0.04, 25.21±0.06mg) and dry weights (92.36±0.01, 24.36±0.01mg) in both Al resistance and sensitive sorghum varieties(Fig.3).

(Fig.4)Effect of various concentrations of Al and SA on relative water content in Sorghum varieties. Data shown are means ± SD (n = 4).

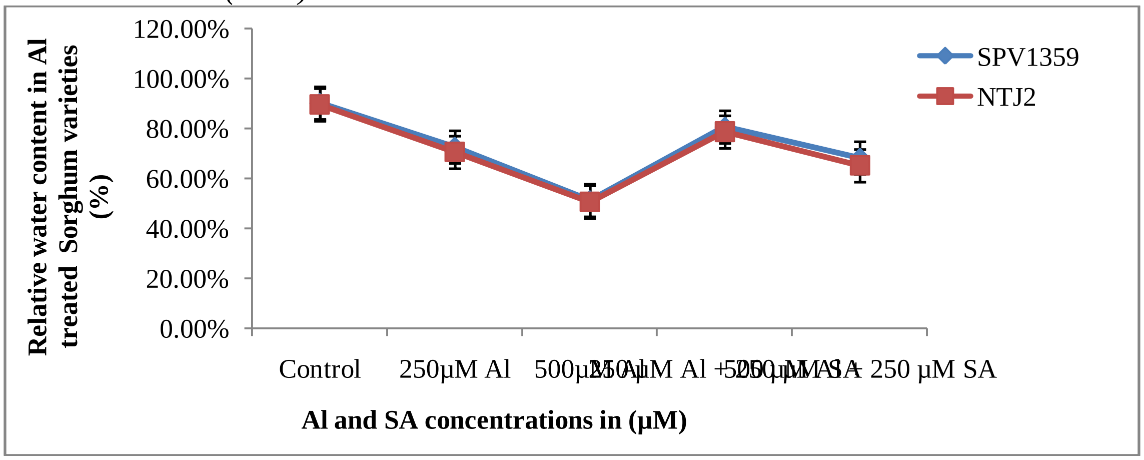

Figure 4: Effect of Al and SA on Relative water content in SPV1359 (Al resistance) and NTJ2 (Al sensitive) Sorghum varieties

Impact various concentrations of Aluminium and SA on Total chlorophyll and Carotenoids content in Sorghum varieties after three day Al treatment. Data are means ± SD (n = 4).

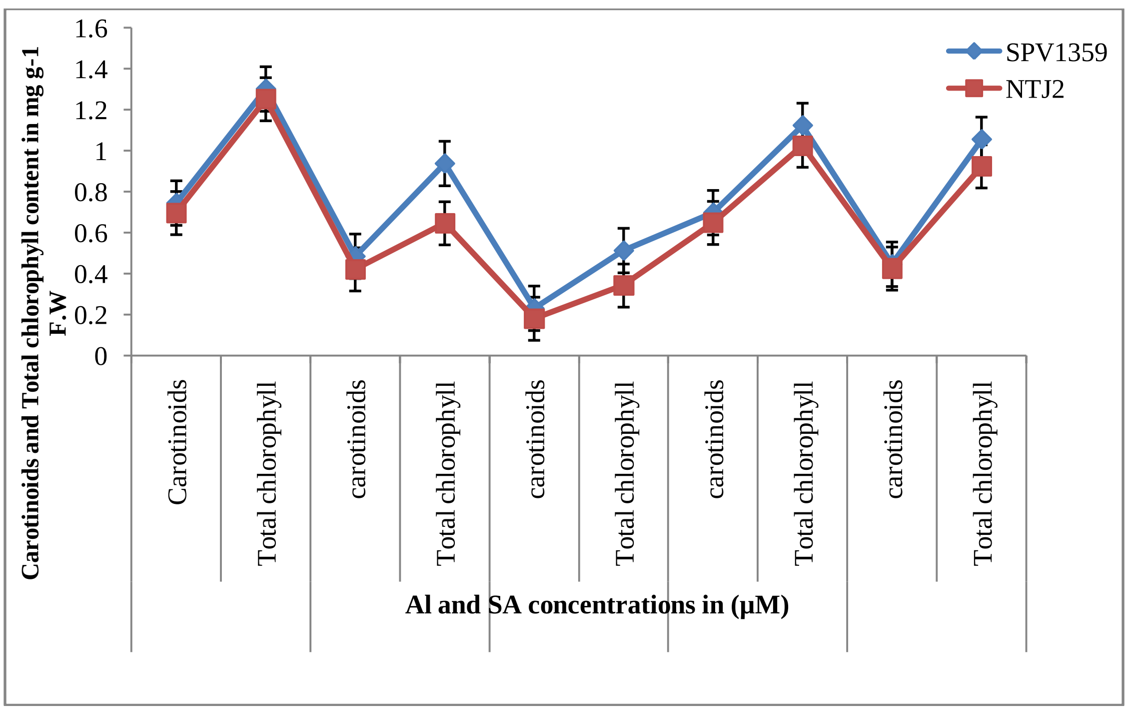

Figure 5: Effect of Al and SA on chlorophyll content in SPV1359 (Al resistance) and NTJ2 (Al sensitive) Sorghum varieties

Al was showed its toxic effect on pigment (chlorophyll and Carotenoids) biosynthesis. In our study the total chlorophyll (0.513±0.12, 0.342±0.13) and Carotenoids (0.231±0.16, 0.18±0.15) content was decreased at higher concentration of Al (500µM) due to the toxic effect of Al and reflect of this Al ions on pigment biosynthesis. From (Fig-6) SA was increased the total chlorophyll (1.056±0.23, 0.924±0.21) and Carotenoids (0.446±0.16, 0.425±0.13) biosynthesis against Al (Fig.5).

DISCUSSION

Germination of seeds is the first physiological evolution affected by various metals (Shankar *et al.*, 2005). One of the Al ion form Al^{3+} inhibits the seed germination rate at different concentrations in white spruce (Nosko *et al.*, 1988), in pigeon pea (Narayanan and Syamala, 1989) and in wheat (Lima and Copeland, 1990). Different concentrations of Arsenic was inhibited the Seed germination rate and seedling growth of in rice (Abedin and Meharg, 2002). In our study seed germination rate was decreased along with increasing concentration of Aluminium and SA treatment improved the seed germination rate against Al (Fig. 1). These seed germination may improve after SA treatment due to the induction of seed germination of physiological process such as hydrolysis, imbibitions, enzymes activation and protrusion according to (Farooq *et al.*, 2008; Sedghi *et al.*, 2010 and Farooq *et al.*, 2009).

The root and shoot length were decreased with increased concentrations of Al by entering in to cytosol of cells and alter the metabolic process after immediate exposure of seedlings to Al (Vazquez *et al.*, 1999; Silva *et al.*, 2000; Sneller *et al.*, 2000 and Abedin and Meharg, 2002). Root growth inhibited plants cannot absorb any nutrients it resulted plants completely decreased its biomass (Mitchell and Barr, 1995). There are some reports are clearly saying that After Al treatment initially Al inhibit the root expansion and elongation behind the cell division (Frantzios *et al.*, 2001). Many researchers investigated the inhibition of seed germination rate, root and shoot lengths and fresh and dry weights up on Al treatment in different plant species (Yang *et al.*, 2007 and Panda *et al.*, 2009).

Chlorophyll

In our experiment after Al treatment the Carotenoids and total chlorophyll content was severely decreased along with the increased of concentration of Al. similar results were observed in some reports With increase in concentration of metal accumulation the total chlorophyll content was decreased according to (Gadallah, 1994 and Sharma and Gaur, 1995). The heavy metals are responsible for reduction of total chlorophyll concentration in plants according to the reports of (Ewais, 1997) used *Cyperus difformis* L.

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