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PLANT PHYSIOLOGY

Study of Growth and Antioxidant Enzymes in *Andrographis paniculata* (Burm f.) Wall ex Nees. as Influenced by Salinity and Alkalinity

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ABSTRACT

An attempt was made to study the influence of salinity (100mM, 150mM, 200mM and 250mM NaCl) and alkalinity (50mM, 100mM, 150mM and 200mM $NaHCO_3$) on Plant height, Number of Leaves, Leaf Area, Relative Water Content, Catalase and Peroxidase activity of *Andrographis paniculata*. Untreated plants were kept as control. The plant samples were analyzed for 100 DAS at every 20 days interval. The results indicated that the reduction in growth parameters were abrupt in *Andrographis* and decreased in above mentioned growth parameters at high salt concentrations (250mM NaCl and 200mM $NaHCO_3$) were observed. Similarly, alteration in the antioxidant enzymes viz. catalase(CAT) and peroxidase (POD) activity was observed in all the treatment. The maximum increment in CAT and POD activity was recorded at 200mM NaCl and 150mM $NaHCO_3$. The results revealed that the extent of alkaline stress induced changes was higher than saline stress and alkaline conditions impose more deleterious effect on *Andrographis* plant.

Highlights

- *Andrographis paniculata* is an important medicinal plant belongs to family Acanthaceae commonly called "King of Bitters" or "Kalmegh".
- Salinity and Alkalinity both decreased the growth of *Andrographis* parallels to increasing concentrations. However, the maximum reduction in growth was observed under alkalinity as compared to salinity.
- An increment in Antioxidative enzymes viz. CAT and POD was observed under both saline and alkaline stress. The maximum increment in CAT and POD activity was recorded at 200mM NaCl and 150mM $NaHCO_3$. However, at high level of salt concentrations (250 mM of NaCl and 200 mM $NaHCO_3$) it was significantly decreased.

Keywords: Salinity, Alkalinity, growth, Antioxidative enzymes, *Andrographis paniculata*

Salinity and alkalinity are mainly caused by the harmful salts which are NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 coming from neutral salts and alkaline salts (Yang *et al.* 2008). Both saline salt stress and alkaline salt stress differ greatly either in their mechanism of action and/or in the mechanism of physiological responses of plants (Guo *et al*. 2010; Rakshit *et al.* 2010). Therefore, they should be called saline stress and alkaline stress respectively (Shi and Yin 1993). Salt stress alters the various biochemical and physiological responses in plants and causes adverse effects on photosynthesis, growth and development (Talei *et al*. 2013; Gupta and Huang 2014). The modification in growth and development of plants caused due to salinity may lead to accumulation and depletion of certain metabolites (Gumi *et al.* 2013). Salt stress generally involves osmotic and ionic stress but alkaline stress involves physiological drought, high pH and ion toxicity and also maintain intracellular ionic balance (Gong *et al.* 2014). Besides this, salt stress also induces oxidative stress, that results in the formation of reactive oxygen species

(ROS) such as superoxide (O_2^-), hydroxyl radical (°OH), hydrogen peroxide (H_2O_2), peroxide ion (O_2^{-2}) and singlet oxygen (O_2) and these are involved in various metabolic processes as well as membrane lipid peroxidation and membrane leakage (Gunes *et al*. 2007; Avinash and Bhavnath 2011). To cope up with the effect of osmotic stress, plants have developed efficient antioxidant machinery having two arms, one is enzymatic component like superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR); and another is non-enzymatic antioxidants like ascorbic acid (AA), reduced glutathione (GSH), α-tocopherol, carotenoids, flavonoids, and the osmolyte proline. These two components work together to scavenge ROS (Das and Roychaudhury 2014).

Alkaline salt stress causes detrimental effects on plant through both salt and pH stress (Cardarelli *et al.*, 2010) and plants under alkalinity also suffer from unavailability of some micronutrients essential to plant growth (Shi and Sheng 2005).

Medicinal plants are important crops (Rehm and Espig 1991). Although the effect of salt stress on traditional crops have been extensively investigated, there is a lack of information in case of medicinal plants (Aghei and Komatsu 2013). Salt stress is one of the most important factors affecting plant growth and the production of secondary metabolites (Nicolova and Ivancheva 2005) and cultivated for their active phytochemicals. Owing to their wild occurrence in diverse environment and high curing value they have been considered to be promising plant (Said-Al Ahl and Omer 2011).

Andrographis paniculata belongs to family Acanthaceae is commonly known as 'King of Bitters' or 'Kalmegh'. It is cultivated because of its well-known medicinal value and it grows well in most soil types thus it is widely distributed (Latto *et al.* 2006). It has been extensively used as traditional medicine in India, China and Southeast Asia. The aerial parts possess most of the medicinal properties and are used to treat snakebites, insect stings, fever, sore throat, cough and stomachache (Okhuarobo *et al.* 2014). *Andrographis paniculata* has a wide range of pharmaceutical properties such as anti-HIV (Basak *et al.* 2006), anti-H1N1 (Ko *et al.* 2006), anticancer (Chun *et al.* 2010) and anti-hepatitis (Dumrongsak *et al*. 2009).

The present work was carried out to give a better understanding of the response of medicinal plant *Andrographis paniculata* to saline and alkaline stress by evaluating different Morpho-physiological aspects viz. Plant height, Number of Leaves, Leaf Area, Relative Water Content and Antioxidant enzymes such as Catalase and Peroxidase activity.

MATERIALS AND METHODS

The uniform and healthy seeds of *Andrographis* were selected and dormancy of seeds were broken by using hot water (80 °C for 2 min). Thereafter, seeds were surface sterilized with 70% ethanol for 3 min followed by thorough washing with distilled water to remove the alcohol. Seeds were then allowed to germinate in growth chamber (Temp. 31±2 °C and Relative Humidity 69-71%). Seedlings emerged after 5-6 days. 10-12 days old seedlings were transplanted in earthenware pots containing sterilized sand. The experiments were arranged in a Completely Randomized Block Design (CRBD) with five replicates.

The plants were treated with varying concentrations of NaCl (100mM, 150mM, 200mM and 250mM) and $NaHCO_3$ (50mM, 100mM, 150mM and 200mM) 30 days after growth. Untreated plants were kept as control. Treatments were repeated at each 5-day interval and Hoagland solution was applied in each pot in equal concentration weekly. All the pots were irrigated with equal amount of water daily to keep sand moist to maintain the salinity and alkalinity of the respective mediums.

Collection of plant samples was done at every 20 days and various growth parameters like Plant height, Number of leaves, Leaf area, Relative Water Content, Catalase and Peroxidase enzyme activity were analyzed from 20 DAS up to 100 DAS [Days After Stress (DAS) corresponds to plant growth after first application of stress which represents 50-130 days of plant growth respectively].

The Leaf Area (LA) was calculated by the method of Singh, (1970) by using following formula:

$$LA = L \times W \times 0.877 \text{ (Constant)}$$

Relative Water Content (RWC) was analyzed by the method of Schoenfeld *et al.* (1988). In determining

RWC, the leaves were surface dried gently with tissue paper. Thereafter leaves were weighed to measure Fresh Weight (FW). The leaves were then soaked in distilled water in a glass plate and were left for 5 hours at room temperature. Thereafter, leaves were carefully blotted with tissue paper prior to determination of Turgid Weight (TW). The leaves samples were then oven dried for 6 hours at 65°C. Dried leaves were then weighed to record Dry Weight (DW). The RWC was calculated by the following formula:

$$RWC = \frac{\text{Fresh weight (FW)} - \text{Dry Weight (DW)}}{\text{Turgid Weight (TW)} - \text{Dry Weight (DW)}} \times 100$$

Catalase [E.C.1.11.1.6] activity was analyzed in leaves by the modified method of Chance and Maehly (1955). For Catalase activity, 200 mg of fresh leaves were cut into narrow strips and were placed in glass vials containing 3 ml of phosphate buffer (pH 6.8). The leaf strips were frozen for 3 hours at -4°C followed by thawing. The reaction was initiated by adding 1.0 ml enzyme extract to 2.0 ml of 25 mM H_2O_2 for 10 min at 37°C in an Incubator. The reaction was stopped by adding 1 ml of Titanic Sulphate and the mixture was centrifuged at 1000 rpm for 15 min. The intensity of yellow color was measured at 410 nm by Spectrophotometer.

Peroxidase [E.C.1.11.1.7] activity was analyzed in leaves by the modified method of Shannon *et al.*, (1966). For Catalase 200 mg of fresh leaves were cut into narrow strips and were placed in glass vials containing 3 ml of phosphate buffer (pH 6.8). The leaf strips were frozen for 3 hours at -4°C followed by thawing. The reaction was initiated by adding 1 ml enzyme extract to assay mixture at 30°C. The assay mixture contained 1ml of 15 mM pyrogallol, 1 ml of 50 mM H_2O_2 and 5 ml of distilled water. The reaction mixture was incubated for 15 min at 25°C. Thereafter, the reaction was stopped by adding 0.5 ml of 5% H_2SO_4. The color formed were measured at 420 nm by Spectrophotometer. The activity of Peroxidase has been calculated in terms of μ.mol H_2O_2 destroyed h^1g^1 fresh weight from Standard Curve prepared from H_2O_2.

Two-way ANOVA was applied to determine the significance of results between different treatments. LSD (Least Significant Difference) value was calculated where F-test was found significant.

RESULTS AND DISCUSSION

The result showed that the saline stress and alkaline stress significantly affected the plant growth. Morphological parameters like Plant height (Fig. 1), Number of Leaves (Fig. 2), Leaf Area (Fig. 3) in *Andrographis paniculata* under saline and alkaline stress were studied.

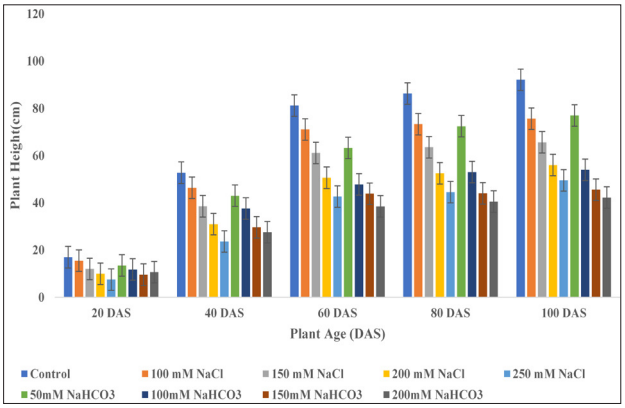

Fig. 1: Effect of Salinity (NaCl) and Alkalinity ($NaHCO_3$) on Plant Height of *Andrographis paniculata*

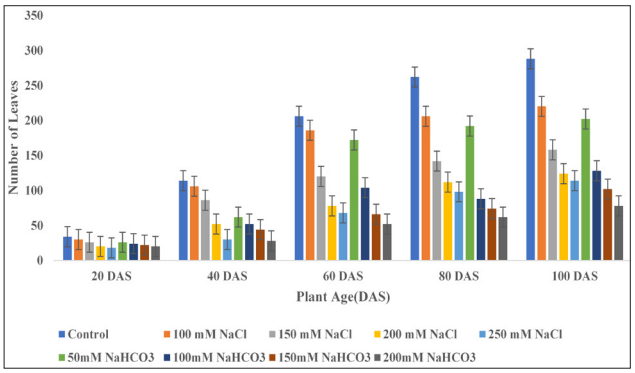

Fig. 2: Effect of Salinity (NaCl) and Alkalinity ($NaHCO_3$) on Number of Leaves of *Andrographis paniculata*

Both saline salt stress and alkaline salt stress caused a significant decrease in plant height, no. of leaves and leaf area for all the treatments of salinity (100mM, 150mM, 200mM and 250mM concentrations of NaCl) and Alkalinity (50mM, 100mM, 150mM and 200mM concentrations of $NaHCO_3$) as compared to control. At low levels of Saline salt (100mM) and Alkaline salt (50mM) plants did not showed distinct variations. However, the plant growth significantly reduced with exposure to higher levels of salinity (150mM, 200mM and 250mM of NaCl) and alkalinity (100mM, 150mM and 200mM of $NaHCO_3$).

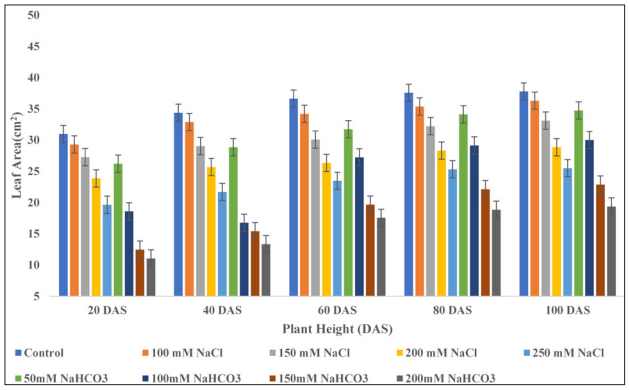

Fig. 3: Effect of Salinity (NaCl) and Alkalinity ($NaHCO_3$) on Leaf area of *Andrographis paniculata*

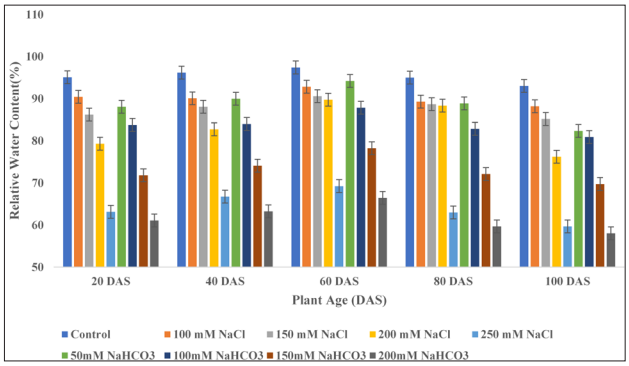

Fig. 4: Effect of Salinity (NaCl) and Alkalinity ($NaHCO_3$) on Relative Water Content (%) of *Andrographis paniculata*

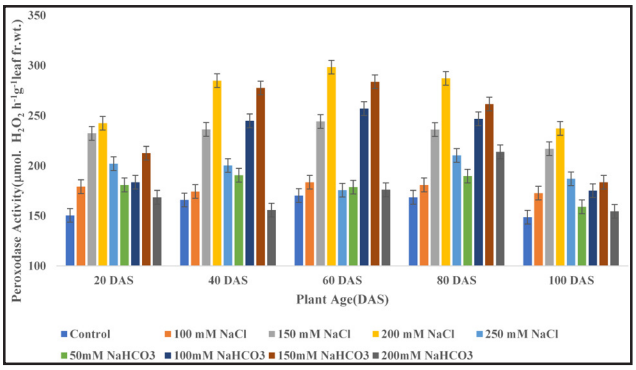

Fig. 5: Effect of Salinity (NaCl) and Alkalinity ($NaHCO_3$) on Peroxidase Activity of *Andrographis paniculata*

In the present study, the reduction in plant height of alkaline salt treated plants were more as compared to saline salt treated plants. This may be due to the fact that increased level of salinity, limits the water absorption and biochemical processes, while, increased level of alkalinity causes a decrease in the uptake of several essential nutrients and increase the pH of soil which directly affects the plant height. This finding is in agreement with previous studies in Cotton plant (Anjum *et al.* 2005); in *Acacia ampliceps* (Mahmood *et al.* 2009); in Gerbera plant (Tavakkoli *et al.* 2016) and in Mungbean plant (Shahi and Srivastava 2016).

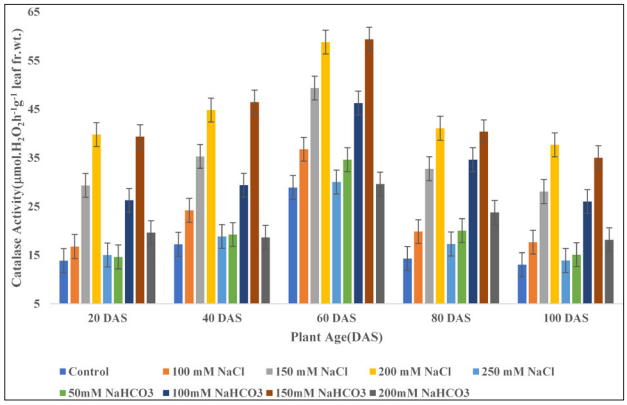

Fig. 6: Effect of Salinity (NaCl) and Alkalinity ($NaHCO_3$) on Catalase Activity of *Andrographis paniculata*

Relative Water Content(RWC), is one of the important indices which showed the water status of plants. RWC is an important physiological parameter to evaluate the plant response against abiotic stresses (Jain and Chattopadhyay, 2010). Under stress conditions plants usually accumulate inorganic ions in vacuoles to decrease the cell water potential. Reduction in leaf RWC indicates loss of turgor that resulted in limited water availability for cell extension process (Katerji *et al.* 1997). Our results (Fig. 4) indicated that at different concentration of salinity (100mM, 150mM, 200mM and 250mM of NaCl) and alkalinity (50mM, 100mM, 150mM and 200mM of $NaHCO_3$) the RWC in *Andrographis* leaves decreased significantly as compared to control. The RWC reduction was less upon exposure to very low concentration of salt treatment, it may be due to the osmotic adjustment of plants under stress conditions.

But a highly significant decrease in RWC, which decreased gradually with increasing salt concentrations. The results showed that the rate of reduction in RWC was greater in alkalinity in comparison to salinity. The greatest reduction in RWC was recorded at 250 mM NaCl and 200mM $NaHCO_3$ treated plant. Similar results were observed in mulberry by (Ahmad and Sharma, 2010) and in tomato by (Mohsenian *et al.* 2012). The results were also in agreement with those of Saneoka *et al.* (2011) and Liu *et al.* (2013) that reported the both saline and alkaline stresses decrease RWC in Foxtail Millet, Proso Millet and in white Swiss chard respectively.

Under stress conditions, plants tended to subvert with Reactive Oxygen Species (ROS) (Bano *et al.* 2013; Kaya *et al.* 2013). To cope up the ROS plants synthesizes various antioxidative enzymes in high amount (Ahmad *et al.*2010, 2011). POD and CAT are the two potent scavengers of ROS. In current study, we found that POD(Fig.5) and CAT(Fig.6) activity increased with increasing saline salt (100mM, 150Mm and 200mM of NaCl) and alkaline salt (50mM, 100mM and 150mM of $NaHCO_3$). However, the enzyme activities did not increase linearly in severely stressed plant (250 mM NaCl and 200mM $NaHCO_3$) as compared to control. The maximum activity of both POD and CAT was recorded at 200mM NaCl and 150 mM $NaHCO_3$. Our findings are in agreement with the studies on Mulberry plant (Ahmad *et al.* 2013), on Sorghum plant (Temizgul *et al*. 2016) and on Mungbean plant (Shahi and Srivastava 2016).

CONCLUSION

From the present investigation, it is evident that high levels of salinity and alkalinity induce stunted growth as well as oxidative damages to the plants by altering the antioxidant machinery leading to disturbance in ion homeostasis, metabolic activities, membrane damage and physiological performance of *Andrographis* plant. Besides, they are also responsible for decrement of water potential and induction of osmotic stress. However, the effect was more pronounced in alkali affected plants as they faced both salt and pH stress.

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