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Research Article

THE EFFECT OF SALT STRESS ON BIOCHEMICALS OF CHILI AT SEEDLING LEVEL

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Abstract

The title of this research is "The Effect of Salt Stress on Biochemicals of Chili at Seedling level of Chili *(Capsicum annuum* L.)". The plant was hydroponically grown in half-strength NaCl solution for 20 days followed by treatments with 0, 50, 100 and 200 mM NaCl for 18 days. Growth parameters of 45-day- old plants were recorded. The plants were harvested and analyzed for the amount of chlorophyll, proline, catalase (CAT) and peroxidase (POD). The low (50 mM NaCl) level of salinity treatment had no deleterious effects on vegetative growth parameters, at higher concentration of NaCl (100 and 200 mm), growth parameters were drastically reduced. Salinity treatments caused a reduction in chlorophyll content, accumulation of proline and enhancement of CAT activity in shoot and root.

Keywords: Salinity, Growth, Activity, Antioxidant enzymes, Chili *(Capsicum annuum* L.).

Introduction

Dry chillies are extensively used as a spice in India. It is sometimes added to tannin or rose gargles for pharyngitis and relaxed sore throats. It is administered in the form of powder, tincture, plaster, ointment and medicated wool etc. *Capsicum* species have been reported to have antioxidant properties.

In India chillies are grown in practically all states occupying about 733,800 ha of land with a production of about 4,39,000t (1971-72) (Kochhar, 2009). Peppers (*Capsicum species*) are economically important crops throughout the world and are mainly used as spices and vegetables. They have gained a lot of medical acclaim as well due to their high ascorbic acid content, vitamin A and medicinally important capsaicins a lot of research is being carried out on various varieties of *Capsicum*. Because of high economic and medicinal importance of the family and its members, it has attracted the attention of morphologists, anatomists, embryologists, physiologists, geneticists, horticulturists and tissue culturists.

Although soil salinisation in the North Region of India is a serious problem for agriculture, Chili can grow well in this area. Therefore, Chili provides us as a good model for studying the mechanisms of plant adaptation with concentrated salt. In the present work, Chili plant being treated with NaCl during vegetative growth stages and the effects of

salinity on some aspects of growth and physiology, including antioxidant enzyme activity, chlorophyll content, proline content, were examined.

Materials and Methods

Seeds of Chili were germinated in distilled water for seven days in a culture room at the Biology SARC Laboratory, Meerut, U.P. during March to May, 2010 under artificial light source (400 ^mol m^{-1} sec^{-1}, 16 h photoperiod) with approximate temperature range between 23- 27°C and 60-89% relative humidity. Seeds were germinated and seedlings were grown in water for 7 days, Chili seedlings at the second-true leaf stage were transferred to 25-l plastic containers containing half-Hoagland solution (Hoagland, 1938) and grown hydroponically in the culture room until the plants were 27 days old. Sodium chloride was then added in small increments until the final concentrations of 0, 50, 100 and 200 mM were reached when the plants were 45-day-old. Nutrient solution was renewed on weekly interval throughout the growing period. The experiment was conducted with three replications.

After 18 days of salinity treatment, the growth, activity of antioxidant enzymes: CAT and POD in shoot and root, proline content in leaves, chlorophyll content in leaves and Na^+ and K^+ contents in shoot and root were determined for the 45-day-old plants.

Growth Parameters

Forty five days old plants were harvested, and plant height and growth parameters were determined as follows: the number of leaves, the leaf area, and the fresh and dry weight of shoots and roots.

Chlorophyll Content

Following extraction of liquid-nitrogen frozen leaf with 80% acetone, the concentration of chlorophyll was determined according to the spectrophotometer method of Porra *et al*, (1989).

Proline Content

Total proline was extracted by the method of Bates *et al*, (1973). Leaf samples (0.1 g) were homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate was filtered through Whatman No. 2 filter paper. Two milliliters of the filtered extract was reacted with 2 ml acid ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 hour at 100 ° C, and the reaction terminated by placing it on ice. The reaction mixture was extracted with 4 ml toluene and vortex. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance was read at 520 nm using toluene as a blank.

Antioxidant Enzyme Activity

Catalase activity was determined by measuring the initial rate of disappearance of hydrogen peroxide as described by Velikova *et al*, (2000). The reaction mixture (3 ml) contained 10 mM potassium phosphate buffer (pH 7.0) and 0.1 ml enzyme extract and the

reaction was started by adding 0.035 ml of 3% hydrogen peroxide. A decrease in hydrogen peroxide concentration was followed by a decline in optical density at the wavelength of 240 nm. The non-enzyme extract mixture served as a blank. The catalase activity was calculated using the extinction coefficient of 40 mM"^{1}cm"^{1} and the activity was expressed as ^mol H_2O_2 reduced mg protein^{-1}min^{-1}.

Peroxidase activity was determined as an increase in optical density due to the formation of guaiacol dehydrogenation product according to Velikova *et al,* (2000). The reaction mixture (3 ml) contained 10 mM potassium phosphate buffer (pH 7.0), 0.04 ml enzyme extract, 0.6 ml guaiacol, and 1% (w/v) aqueous solution, and the reaction was started by adding 0.15 ml of 100 mM hydrogen peroxide. The absorbance was recorded at the wavelength of 470 nm in a spectrophotometer. The non- enzyme extract mixture served as a blank. The peroxidase activity was determined by using the extinction coefficient of 26.6 mM^{-1}cm^{-1} and the activity was expressed as prnol GDHP mg protein^{-1}min^{-1}.

Statistical Analysis

The experimental design was a randomized complete block. The data are presented with the respective standard errors of means and the least significant difference (LSD 0.05) between treatments, derived from analysis of variance.

Results

Growth Parameters

Salinity treatment caused the retardation in growth and development of Chili plant The effects of salinity treatments on the vegetative growth parameters of 45-day-old plant after 18 days of NaCl treatment are summarized in Table 1. Treatment with 50 mM NaCl resulted in a non-significant reduction in number of leaves, leaf area, root height as well as fresh and dry weight of shoot and root. Higher concentrations of 50 and 100 mM NaCl caused 28.95% and 39.47% reductions in number of leaves and 21.62% and 30.95% reductions in leaf area respectively. Salinity treatment at 50 and 100 mM had less effect on the height of shoots (7.88% and 13.70% reduction, respectively) than on those of roots (17.68% and 21.80% reduction, respectively).

The weight of fresh shoot was drastically reduced from 5.07 to 4.30 (15.13%) and 4.10 (19.08%) g when treated with 50 and 100 mM NaCl respectively. whereas the opposite outcome of the root, was observed. On a dry weight basis, NaCl at 50 and 100 mM had more deleterious effects on shoot growth (6.77% and 12.78% reduction, respectively) than root growth (30% and 50% reduction, respectively).

Table 1: Effects of NaCl on vegetative parameters of 45-day-old Chili plants after 18 days salinity treatment: number of leaves (leaf/plant), leaf area (cm^2), plant height (cm), fresh and dry weight of shoot and root (g/plant).

NaCl (mM)	Number of leaves (leaf)	%	Leaf Area (cm^2)	%	Shoot height (cm)	%	Root height (cm)	%	FW of shoot (g)	%	DW of shoot (g)
0	12.67+0.5[a]	0	15.96+1.90[a]	0	29.20+3.70[a]	0	27.53+2.82[a]	0	5.07+0.38[a]	0	0.44+0.02[a]
50	11.00+1.00"	-13.16	15.52+1.37[a]	-2.76	29.13+4.21[a]	-0.23	27.10+0.35[a]	-1.57	5.03+0.15[a]	-0.66	0.44+0.01[a]
100	9.00+1.00[b]	-28.95	12.51+1.53[b]	-21.62	26.90+1.65[a]	-7.88	22.67+2.99[b]	-17.68	4.30+0.20[b]	-15.13	0.41+0.01[b]
200	7.67+1.15[b]	-39.47	11.02+1.82[b]	-30.95	25.20+2.72[a]	-13.70	21.53+2.20[b]	-21.79	4.10+0.17[b]	-19.08	0.39+0.01[c]

Means in the same column followed by different letters differ significantly at $P<0.05$

Chlorophyll Content and Proline Content

Salinity stress induced changes of several physiological parameters in mature leaves of 45-daysold plants after 18 days of NaCl treatment. Leaf chlorophyll was significantly reduced in stressed plant subjected to 100 mM NaCl compared to the non-stressed plant.

Antioxidant Enzyme Activity

According to investigation of the activity of anti-oxidative enzymes, CAT and POD, it was shown that high concentration of NaCl affected the activities from both of these enzymes. It is apparent that in Chili leaves the activity of CAT was enhanced into a higher extent than POD when the plants are subjected to NaCl stress. CAT activity increased under NaCl treatment in both of shoots and roots comparing with the control one. The plants were treated with low concentration of NaCl, POD activity was significantly decreased in both of shoots and roots and returned to the normal level when treated with higher NaCl concentration, however, was not significantly increased in higher concentration of 100 and 200 mM NaCl caused 15.95% and 14.13% increase in shoot and 5.30% and 5.85% increase in root, respectively.

Discussion

Sort term treatment (18 days) of NaCl at low concentration (25 mM NaCl) to chili plant had little effect on the vegetative growth of 45-day-old chili plant. Higher concentrations ofNaCl (100 and 200 mM) resulted in significant reduction on most of vegetative growth parameters, including Number of leaves, Leaf area, Shoot and root height, fresh and dry weight of shoots and roots. Root growth, as indicated by percentage reduction in dry weight, was likely to be more affected than shoot growth. Similar observations were reported by several authors and reviews by Cuartero and Fernandez- MuHoz (1999).

A decrease in photosynthetic pigment content of Chili plants under high concentration of salt was observed. There was a decrease of 12.5%, and 4.06% of chlorophyll in response to the 100 and 200 mM NaCl treatment, respectively, when compared to the control (Figure 1a). The results obtained in this study are in agreement by those of Jaleel *et al,*

(2008) and Al-Sobhi (2006). Reduction in chlorophyll concentrations is probably due to the inhibitory effect of the accumulated ions. (Ali, 2004).

Salinity treatments caused the increased proline content in Chili plant. The accumulation of nitrogen-containing compatible solutes including proline is known to function in osmotic adjustment, protection of cellular macromolecules from damage by salts, storage of nitrogen and scavenging of free radicals. Many plants, both halophytes and glycophytes, accumulate proline as a non-toxic and protective osmolyte under salinity, including mangrove (Parida *et al*, 2002), maize (Cicek and Cakirlar, 2002), sorghum (de Lacerda *et al*, 2005) and mulberry (Harinasut *et al*, 2003). Some authors have, however, argued that excessively high levels of proline accumulation may be a response to leaf damage when exposed to high NaCl concentration and that a higher level of proline accumulation is associated with salt sensitive traits in Chili (Bolarin *et al*, 1995) and sorghum (de Lacerda *et al*, 2005). Proline accumulation in response to lower salt concentration may contribute positively to salt tolerance, whereas the high concentration in leaf tissues under high salinity treatment may be partly due to leaf damage.

An important consequence of salinity stress is the generation of excessive reactive oxygen species (ROS) which leads to cell toxicity, membrane dysfunction and cell death. Chili plant has been reported to defend against the ROS by enhancement of anti-oxidative enzymes including POD, hydrogen peroxide, superoxide dismutase, Ascorbate peroxidase and glutathione reductase (Lee, 2006). In this Chili cultivar under our experimental condition, CAT played more active roles than POD in plant cells from oxidative stress. The activities of CAT increased with the increase of the concentration of NaCl in shoots and roots of Chili. Reported the enhanced activity of anti-oxidative enzymes (CAT and SOD) in *chili* grown under salt stress (0, 100, 200, 400 mmol/l) NaCl concentration (Li, 2008).

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