Tobacco responds to salt stress by increased activity of antioxidant enzymes

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Abstract

In order to understand the response of tobacco to salt stress, antioxidant enzyme activities, plant biomass and ion content were analyzed in two oriental tobacco genotypes (Basma 31 and SPT 406). Tobacco plants were exposed to 0, 50, 100, 150 and 200 mM NaCl for 12 days. The fresh and dry weight as well as shoot and root length of Basma 31 were greater than those of SPT 406 under increasing salt stress. On exposure to NaCl, SPT 406 showed a higher Na/K ratio than that of Basma 31. Activity of catalase, ascorbate peroxidase and guaiacol peroxidase enzymes increased with increasing NaCl content in leaves of two genotypes, but activities of three enzymes in leaves of Basma 31 were higher than SPT 406. Activities of catalase and ascorbate peroxidase in roots of Basma 31 and SPT 406 increased with increasing external NaCl concentration up to 150 and 100 mM, respectively. The highest guaiacol peroxidase activity in roots of Basma 31 and SPT 406 was observed in 100 and 50 mM NaCl, respectively. These studies established that Basma 31 was able to tolerate higher salinity in comparison with SPT 406. Our results suggested that catalase, guaiacol peroxidase and ascorbate peroxidase play a major role in estimating the salt stress tolerance of tobacco genotypes.

Keywords: tobacco; antioxidant enzyme; ion content; salt stress; tolerance

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Introduction

Salinity is one of the important environmental stresses that adversely affects growth and productivity of plants throughout the world (Zhu, 2001; Parviz and Satyawati, 2008). It is mainly related to increase in Na^+ and Cl^- ions and decrease in K^+ and Ca^+ ions in plants (Perez-Alfocea et al., 1996; Al- Karaka, 2000; Shilpim and Narendra, 2005). Many plants in response to salinity, accumulate sodium and chloride ions in their vacuoles or diffuse it to different parts of the plant. In this way, the cytoplasmic water potential of plants is protected from the adverse effect of salinity (Munns, 2002). The primary effects of salinity such as hyperosmotic and ion imbalance can cause oxidative stress.

Salinity can cause negative effects on plant metabolism such as ion distribution and toxicity, reduced water potential and decreased level of CO_2 fixation (Bohnert and Jensen, 1996). Different environmental stresses such as salinity,

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thermal stress and selenium (Hernandez et al., 2000; Sudhakar et al., 2001; Chen et al., 2008) causes generation of reactive oxygen species.

Plants, similar to other aerobic organism, are equipped with efficient ROS- scavenging mechanism which includes natural antioxidants such as carotenoids, glutathione, ascorbate, tocopherols, and enzymatic antioxidant systems such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) (Allen, 1995; Mittler et al., 2004). These mechanisms are expressed constitutively to cope with ROS formed under normal conditions. It is possible to find a part of the antioxidant defense system in different subcellular compartments (Hernandez et al., 2000).

In this study, two genotypes of tobacco were investigated for the effects of salt stress. Na^+ and K^+ ions content, physiological characteristic and activity of antioxidant enzymes were measured in leaves and roots. A comparison of the response of these genotypes to salinity can help elucidate the mechanisms responsible for salt tolerance in tobacco.

Materials and Methods

Plant materials and growth conditions

Seeds of tobacco (*Nicotiana tabacum*) were planted in polystyrene trays (57 × 32 cm) containing perlite and peat moss (ratio of 50:50). Five weeks after germination, tobacco seedlings were shifted to Hoagland solution in pots (2 L), when plants averaged about 12 cm in height. After three weeks, plants with 4-5 leaves were exposed to NaCl (0, 50, 100, 150 and 200 mM NaCl) in Hoagland solution (1/2 strength) for 12 days. Plants were grown in controlled-environment chamber with a 16/8 h day/night light cycle, 25 ˚C and 70% relative humidity. Twelve days after the treatment, shoots and roots were weighed and dried at 70 ˚C for 48 hours. For enzyme assay the root and leaf tissues were stored at -80 ˚C until used.

Determination of Na^+ and K^+ contents

Plant materials were dried at 70 ˚C for 48 h, dissolved in 10 ml distilled water and incubated in a water bath (100 ˚C) for 1 h. The extracts were filtered through Wattman filter paper and filtrates were analyzed for Na^+ and K^+ contents using a flame photometer (405, Fater electronic).

Enzyme extraction and activity assay

All of the following experiments were performed at 4 ˚C. A portion (0.5 g) of leaf and root was rapidly crushed in 5 ml sodium phosphate buffer (pH= 7.8, 50 mM) containing 2 mM ascorbic acid, 1 mM sodium EDTA and 2% (W/V) polyvinylpyrrolidone. After centrifugation (6000 g, 30 min), the supernatant was collected and used for CAT (catalase), GPX (guaiacol peroxidase) and APX (ascorbate peroxidase) activity assays.

CAT (EC= 1.11.1.6) activity was assessed based on the method of Bergmeyer (1970). The reaction mixture comprised sodium phosphate buffer (pH= 7.0, 50 mM), H_2O_2 (%1) and 50 µl enzyme extract. The subsequent consumption of H_2O_2 was measured at 240 nm for 1 min (E= 0.036 mM per cm). One unit of CAT activity was defined as the amount of enzyme needed to reduce 1 mM of H_2O_2 per min.

GPX (EC= 1.11.1.7) was assayed in a reaction mixture comprising phosphate buffer (pH= 6.0, 50 mM), H_2O_2 (10 mM), guaiacol (2.25 mM) and 50 µl of enzyme extract. The subsequent increase in absorbance of oxiguaiacol was measured at 470 nm and was defined as µmol of H_2O_2 per min (Scebba et al., 2001).

APX (EC= 1.11.1.11) activity was estimated in a reaction mixture that comprising potassium phosphate buffer (pH= 7.0, 50 mM), H_2O_2 (0.1 mM), ascorbic acid (0.25mM), EDTA- 4H (0.1mM) and 50 µl of enzyme extract. The subsequent reduction in ascorbic acid was estimated at 290 nm for 1 min (E= 2.8 mM per cm). A unit of APX is expressed as the amount needed to oxidize 1 mM of ascorbate per min (Nakano and Asada, 1987). All chemicals and reagents were purchased from Merk Chemical Co. (Germany).

Statistical analysis

All data analyses were performed using SPSS software version 16. Results were analyzed

using one- way ANOVA (analysis of variance) followed by Tukey's multiple comparison test. Significance level was determined at $p< 0.05$ and the results were expressed as mean values and standard error (SE) of the mean and all the assays were carried out in triplicate.

Results

Growth parameters

The lengths, fresh and dry weights of shoot, fresh and dry weights of root in both genotypes were measured after exposing the plants to 0, 50, 100, 150 and 200 mM NaCl for 12 days (Table 1). High salinity stress (200 mM NaCl) resulted in a 62% and 67% decrease in shoot length of Basma 31 and SPT 406 respectively compared to untreated plants. The root length of Basma 31 and SPT 406 was decreased by 55% and 59% at 200 mM NaCl, respectively (Table 1).

The results showed that fresh and dry weights of shoot significantly was affected by salinity levels ($p< 0.05$). In Basma 31, high salinity (150 and 200 mM NaCl) caused 64% and 85% decrease in fresh weight of shoot, and 69% and 78% reduce in dry weight of root (Table 1). Whereas this decrease was more severe in SPT 406 than Basma 31 so that at salinity levels 150 and 200 mM NaCl reduced fresh weight of shoot by 77% and 91%, and dry weight of shoot by 72% and 87%, respectively (Table 1). In low NaCl concentration (50 mM), the fresh and dry weights of root showed no significant changes, however they decreased with increasing NaCl concentration (200 mM) (83% and 73% in Basma 31 and 91% and 81% for SPT 406 respectively) (Table 1).

Na^+ and K^+ contents

The Na^+ content of the leaves and roots of both Basma 31 and SPT 406 significantly increased in treated plants (Fig. I). The Na^+ content of the leaves of SPT 406 was higher than that of Basma 31, whereas Na^+ content in the roots was lower. In both genotypes, salt treatment had a negative effect on K^+ content. The decrease in K^+ content was more severe in roots than in leaves, where the K^+ content of leaves at 200 mM NaCl decreased by 55% in SPT 406 and by 48% in Basma 31, whereas in roots it decreased by 93% and 92% in SPT 406 and Basma 31, respectively (Fig. I).

At all NaCl concentrations, SPT 406 showed higher Na^+/K^+ ratio compared to those of Basma 31, so that the leaves Na^+/K^+ ratio increased by 5.7- fold and 4.3- fold in SPT 406 and

Table 1
The lengths of root and shoot (cm), and fresh and dry weight of roots and shoots (g), Na^+/ K^+ ratio of shoot and root in two genotypes (Basma 31 and SPT 406) of tobacco (*Nicotiana tabacum*) at different concentrations of salinity (0, 50, 100, 150 and 200 mM NaCl)

Genotype	Salinity (Mm NaCl)	Shoot Lenght	Root Lenght	Shoot FW	Root FW	Shoot DW	Root DW	Shoot Na^+/ K^+	Root Na^+/ K^+
Basma 31	0	39.9a	26.5a	29.1a	5.31a	1.47a	0.151a	0.02a	0.08a
	50	30.5a	21.9b	18.9b	5.12a	1.29b	0.146a	0.63b	1.29b
	100	24.6b	16.7c	13.4c	3.21b	0.68c	0.093b	2.57c	4.93c
	150	19.4c	13.6d	10.4c	1.53c	0.43d	0.065c	3.14d	13.5d
	200	15.1d	11.9d	4.26d	0.92d	0.31e	0.040d	4.28e	30.6e
SPT 406	0	41.06a	21.7a	29.4a	3.29a	1.36a	0.132a	0.05a	0.17a
	50	29.03b	18.8b	17.1b	2.91a	1.14b	0.091b	1.07b	2.04b
	100	25.06c	13.9c	12.5c	1.73b	0.73c	0.076c	2.72c	5.42c
	150	21.6d	11.5c	6.72d	1.15c	0.37d	0.054d	4.13d	16.4d
	200	13.6d	8.9d	2.56e	0.28d	0.17e	0.026e	5.75e	50.9e

Results are the means ± standard Error (n= 3). Different

Basma 31 at 200 mM NaCl, respectively. Also, the roots Na^+/K^+ ratio increased by 50.9- fold and 30.7- fold in SPT 406 and Basma 31 at 200 mM NaCl, respectively (Table 1).

Antioxidant enzyme activity

Interestingly, Basma 31 showed significantly higher antioxidant enzymes activities than those in SPT 406 under salt stress conditions. The NaCl dose dependency of CAT activity was observed in the leaves of both tobacco genotypes. In 50 and 100 mM NaCl, the CAT activity increased by 5.8% and 44% in Basma 31, and by 3.9% and 25% in SPT 406, respectively. At 150 and 200 mM NaCl, the CAT activity in both genotypes decreased (Fig. II). In comparison to untreated plants, the CAT activity in roots of both genotypes increased at all levels of salinity but the maximum CAT activity was observed at 200 and 150 mM NaCl concentration in Basma 31 and SPT 406, respectively (Fig. II).

There was a gradual increase in GPX activity of leaves of Basma 31 with increasing salinity. However, Basma 31 showed over twofold more GPX activity than untreated plants at 200 mM NaCl. SPT 406 showed less increase under similar conditions and maximum activity of the enzyme was observed at 150 mM NaCl (Fig. II). In roots, the maximum GPX activity was observed at 100 and 50 mM NaCl concentrations in Basma 31 and SPT 406, respectively. At 200 mM NaCl, GPX activity decreased by 42% in Basma 31 and 63% in SPT 406 (Fig. II).

APX activity significantly increased with increasing NaCl concentration in leaves of both genotypes, so that APX activity increased by 7.7-fold in Basma 31 and 6.3- fold in SPT 406. In roots, APX activity showed a strong increase at 150 mM NaCl (6.6- fold higher compared to untreated plants) in Basma 31, whereas maximum APX activity in SPT 406 was observed at 100 mM NaCl (3.6- fold higher compared to untreated plants) (Fig. II).

Discussion

A gradual decrease was observed in lengths and fresh and dry weights of shoot and root in Basma 31 and SPT 406 genotypes under

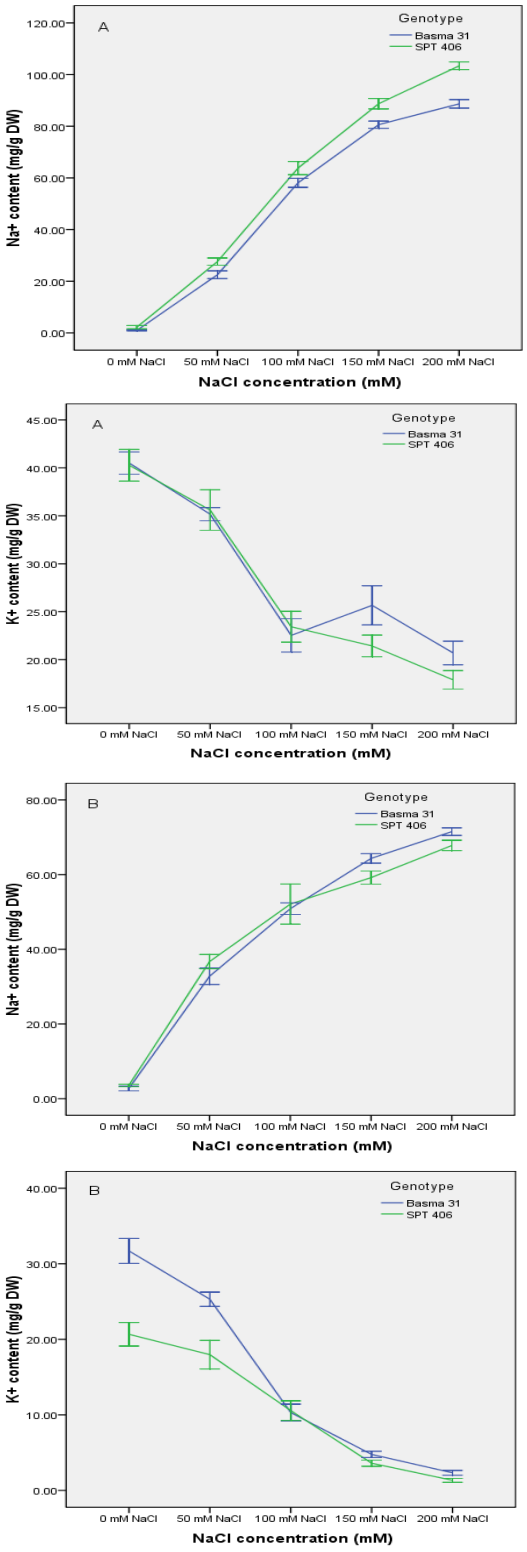

Fig I. Effect of salt stress on the Na^+ and K^+ contents of leaves (A) and roots (B) at different salinity levels (0, 50, 100, 150, and 200 mM NaCl). The experiments were repeated tree times and the results show the average ± standard error that is denoted by error bars (One Way ANOVA, DW= dry weight).

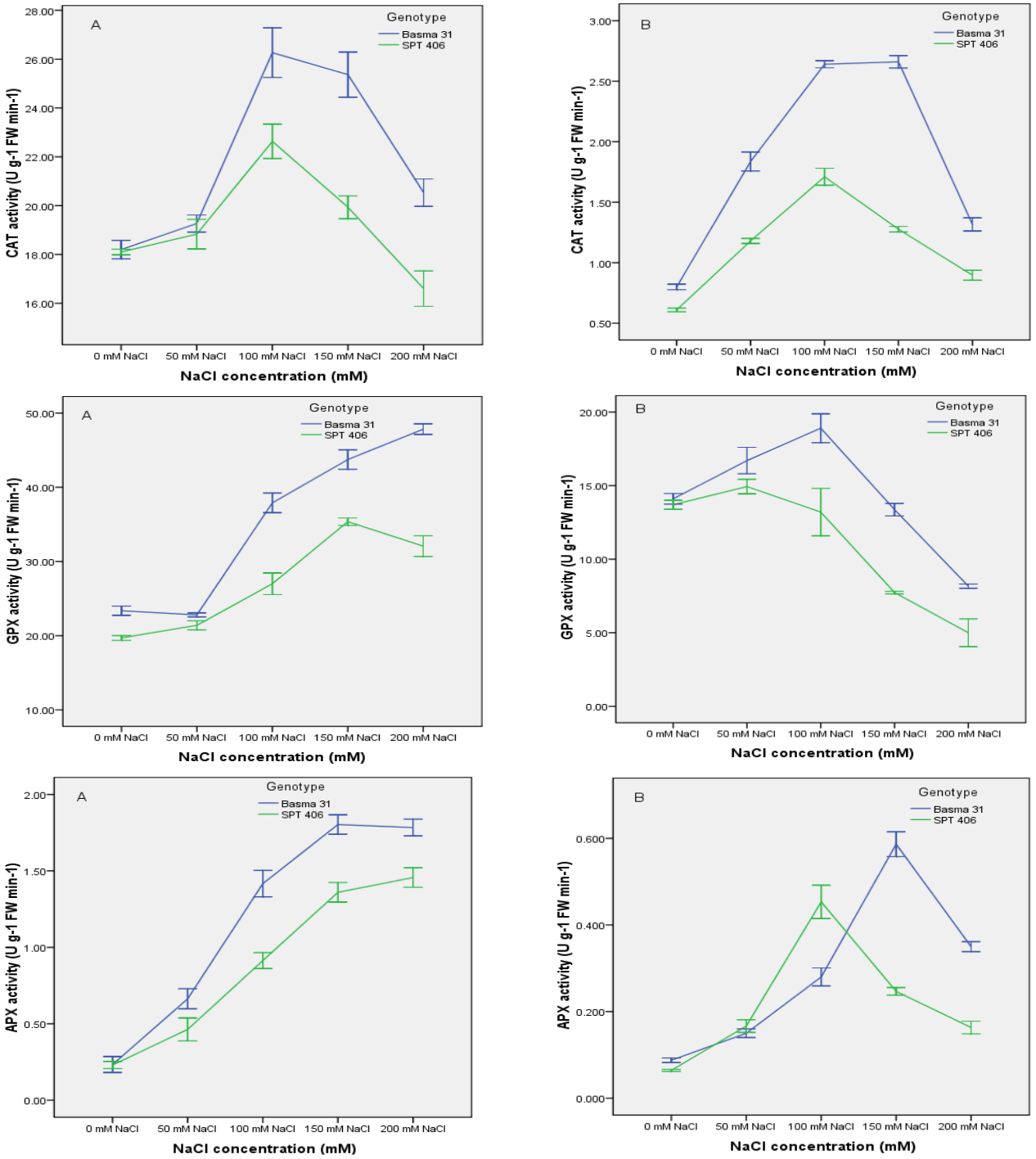

Fig II. Effects of salt stress on the activity of antioxidant enzymes. The activities of catalase (CAT), guaiacol peroxidase (GPX) and ascorbate peroxidase (APX) were measured in leaves (A) and roots(B) 12 days after treatment. The experiments were repeated three times and the results show the average ± SE that is denoted by error bars (One Way ANOVA, FW= fresh weight).

salinity stress. However this negative effect was more severe in SPT 406 than in Basma 31. So that Basma 31 can be considered as a relatively more salt- tolerant genotype than the other genotype. Several studies consistent with our results, salt-sensitive genotypes of lentil (Bandeoglu et al., 2005), potato (Aghaei et al., 2009) and tobacco (Celik and Atak, 2012) showed significant growth decrease compared to tolerant genotypes under salt stress.

A reduction in K^+ concentration and increase in Na^+ concentration under salinity

condition, as identified in this study, was reported in soybean (Liu and Staden, 2001), rice (Gao., et al., 2007), potato (Aghaei et al., 2009) and canola (Bybordi et al., 2010). Higher concentration of Na^+ due to salt stress, leads to cell dehydration and membrane dysfunction. This osmotic and ion imbalance could inhibit important metabolic pathways and cause plant growth reduction. Na^+/K^+ ratio increased in SPT 406 compared to the Basma 31. The results obtained from this study suggested that increased salt stress results in Na^+ toxicity and K^+ deficiency in leaves of plants which might, after functional state, resulting physiological stress.

Environmental stresses such as salt stress induce reactive oxygen species (ROS) generation that causes oxidative damage to proteins, nucleic acids and membranes (Gomez et al., 1999). To limit damage to cells by reactive oxygen species, plant cells employ antioxidant enzymes including CAT, GPX and APX (Mittler, 2002). The present results indicated that under salt stress, a significant increase in CAT and APX activities was observed in both tobacco genotypes. Although CAT activity in leaves and roots and also APX activity in roots decreased at high concentration of salinity, they remained as high as those present in control plants. Similar APX and CAT activities induced by salinity have also been reported in salt- tolerant sugar beet (Bor et al., 2003), rice (Vaidyanathan et al., 2003), plantain (Sekmen et al., 2007), potato (Aghaei et al., 2009), canola (Bybordi et al., 2010) and tobacco (Celik and Atak, 2012) which were consistent with the results obtained in this study. The results also showed that GPX activity in leaves of two genotypes increased under salt stress. Increasing of GPX activity was also observed due to salinity in mulberry (Harinasut et al., 2003), soybean (Aghaleh and Niknam, 2009; Weisany et al., 2012), pea (Shahid et al., 2011) and tobacco (Celik and Atak, 2012). Although both genotypes showed increases in GPX activity in leaves, their roots showed decrease in GPX activity under salt stress.

The differences in plant growth and antioxidant activities, and also Na^+/K^+ ratio during NaCl treatment in two tobacco genotypes under study may be associated with differences in tolerance to salinity stress. Therefore, Basma 31 with less decrease in growth, less Na+/K+ ratio and higher antioxidant activity compared to SPT 406, showed a high capacity to tolerate salinity stress.

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