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Full Length Research Paper

Effects of NaCl stress on seed germination, early seedling growth and physiological characteristics of cauliflower (*Brassica oleracea* L. var. *botrytis* L.)

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Effects of salt stress on seed germination, early seedling growth and some physiological characteristics were evaluated for four cauliflower species in seven treatments of salinity including 0 (control), 34, 68, 102, 136, 170 and 204 mM NaCl in a three replicated randomized completely block design (RCBD). This result shows that different treatments of salinity had considerable effect on the germination percentage, vigor index, seedling height, root length, peroxidase (POD), superoxide dismutase (SOD), catalase (CAT) and root activities, malondialdehyde (MDA), soluble protein, chlorophyll and carotenoid contents. Germination percentage and vigor index of four species showed considerable decrease with increasing salinity up to 204 mM NaCl except for germination at 34 mM NaCl. The seedling growth was significantly affected by all salinity levels. Particularly at 136 mM and 170 mM NaCl, seedling height and root length of all species were reduced significantly. POD and root activities of all four species were reduced gradually as salinity increased. SOD activities and chlorophyll and carotenoid contents of these species increased first at 34, 68 and 102 mM NaCl and then decreased at 136 and 170 mM NaCl. Both CAT activity and soluble protein content showed the reversed changing trend with SOD etc. MDA Contents of these four species showed considerable increase with increasing salinity up to 204 mM NaCl.

Key words: Salt stress, cauliflower, germination, physiological characteristics, antioxidative enzyme, MDA.

INTRODUCTION

Salt stress is a major environmental stress, which affects seed germinating, plant growth and development, metabolic processes and productivity (Prado et al., 2000; Pujari and Chanda, 2002; Al-Taisan, 2010). Because of improper irrigation, fertilization and soil management, salinity is becoming more prevalent and worldwide, and has influenced the productivity of agricultural system (Gulen et al., 2006). Therefore, understanding the physiological mechanisms of salt stress is essential and useful for evaluating of salt tolerant cultivars and expanding planting in salinity soils.

Crop salinity sensitivity varies with species, genotypes and growth stages (Prado et al., 2000; Pujari and Chanda, 2002). Salt stress induced inhibition of seed germination, seedling growth and metabolic processes were reported in maize (Azevedo et al., 2004), wheat (Brini et al., 2009), safflower (Demir et al., 2003), cotton (Diego et al., 2003), sunflower (Mehmet et al., 2006), etc. In cauliflower, Jamil et al. (2005) studied on salinity tolerance of *Brassica* species at germination and early seedling growth, and found that seed germination, germination rate, shoot and root length, shoot and root fresh weight were considerable decrease with increasing salinity. However, the result they got was from only one cauliflower cultivar, which might be no general conclusion.

In this study, four cauliflower species were used to

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Abbreviations: SOD, Superoxide dismutase; **POD**, peroxidase; **CAT,** catalase; **MDA,** malondialdehyde.

investigate the influence of salinity on germination, early seedling growth, major antioxidative enzymes activities, soluble protein and photosynthesis pigment contents, etc. during early seedling growth.

MATERIALS AND METHODS

Plant materials and salt stress conditions

Seeds of four cauliflowers YN, SF, SL and YD collected from the Experiment Farm of Wenzhou Academy of Agricultural Science in 2009 were used in this study.

For each treatment per replication, 100 Seeds samples were sterilized in solution of 75% ethanol for 15 min, then washed with distilled water and blotted up with absorbent paper. Then, they were germinated on filter paper in Petri dishes in controlled conditions (20 to 25°C, 8 h light /16 h dark, and 37.5 $\mu molm^{-2}s^{-1}$ photosynthetic active radiations) for seven days. One milliliter salt solution of different concentrations, that is, 0, 34, 68, 102, 136, 170 and 204 mM was added into the Petri dishes every day for salt stress, respectively. The experiment was arranged in a randomized completely block design with three replicates.

Parameters for germination and early seedling growth

Number of germinated seeds was recorded every day. In the seventh day, germination percentage (GP) was calculated, seedling height and root length were measured (10 plants each treatment). Vigor index was calculated as $VI = S \times (G_t/D_t)$ (Zhu et al., 2010a), where *S* is root length of the seventh day, G_t is the number of germinated seeds in the "t th" day, D_t is number of days from the first day to the "t th" day.

Activities of SOD, POD, CAT and content of MDA

A leaf sample (0.5 g) was ground in a mortar (pre-cold in -20°C icebox) and homogenized in 6 ml of 50 mM phosphate buffer (pH 7.0) followed by centrifuging at 15000 × g for 20 min at 4°C (Zhu et al., 2010a).

The supernatant was collected and used for assays of SOD, POD, CAT and MDA as described by Azooz et al. (2009). All the enzyme activities were calculated and expressed as $U \cdot min^{-1} \cdot g^{-1}$ DW (drougth weight). MDA content was calculated and expressed as $nmol \cdot g^{-1}DW$. All the physiological characteristics in this study were also assayed with six replicates for each treatment per replication.

Contents of chlorophyll and carotenoid

A leaf sample (0.5 g) were washed by tap water for 3 min, blotted up with absorbent paper, and then soaked in extracting solution (ethanol: acetone: H_2O = 9 : 9 : 2) for three days in dark at 25°C. Then, the supernatant was used for the determination of Chlorophyll and carotenoid following the protocol described by Tang et al. (2004). All contents of pigment were calculated and expressed as $mg \cdot g^{-1}DW$.

Content of soluble protein

A leaf sample (0.5 g) was homogenized with 50 mM phosphate buffer (pH 7.0) containing 2% polyvinylpyrrolido (PVP) followed by centrifuging at 7379 × g for 15 min at 4°C. The supernatant was collected and used for assays of soluble protein content describe by Zhu et al. (2010b). Contents of soluble protein were calculated and expressed as $mg \cdot g^{-1}DW$.

Root activity

Root activity was determined mainly as describe by Sheng et al. (1998). Samples of root (0.5 g) were extracting 4 h at 37°C darkly in solution (10 ml) which contained 0.4% triphenyltetrazolium chloride (TTC) and 50 mM phosphate buffer (pH 7.0). Then, the roots were taken out and put in 10 ml ethanol and extracted for 3 h in a water bath at 80°C. The supernatant was placed in a quartz cuvette and optical density was recorded by Unico UV-2802PC spectrophotometer (Unico American Instrument Co., Ltd.) set at 485 nm. Root activity was determined by measuring the difference in optical density and expressed as $A485 \cdot g^{-1}DW$.

Statistical analysis

The experiment was design in a randomized completely block design (RCBD) with three replicates. All data were subjected to analysis of variance (ANOVA) using Microsoft Excel 2003 and DPS 9.50 software. Percentage data were arcsine-transformed before analysis according to:

$$\hat{y} = \arcsin \sqrt{x/100}\,.$$

Significant level, P = 0.05 were used.

RESULTS

Effect of NaCl stress on germination and early seedling growth

Table 1 shows that seed germination of YN, SF, SL and YD was affected differently by salt treatments. Increased salt concentration caused a decrease in germination percentage except for 34 mM NaCl. Vigor index was almost declined significantly with the induction of salinity concentrations.

The reduction of germination percentage and vigor index was strongest particularly at highest levels of salt concentration compared to the control.

Influence of salt stress on seedling height and root length during early seedling growth varied with NaCl concentrations (Table 2).

Compared with the controls, seedling height of four species were increased considerably at 34 mM and 68 mM NaCl, while inhibited severely as NaCl concentration raised from 102 mM to 170 mM (Table 2).

The data on average root length of all species showed a strong inhibition with increasing level of salt solution (Table 2). The inhibition of seedling growth of all species was strongest especially at highest levels of salinity as compared with the control.

Effect of NaCl stress on POD, SOD, CAT activities and MDA content

Effects of salinity levels on POD, SOD and CAT activities,

Table 1. Effect of NaCl stress on germination percentage and vigor index.

NaCl (mM)	Germination percentage (%)				Vigor index			
	YN	SF	SL	YD	YN	SF	SL	YD
0	98.67[a]	99.33[a]	92.00[a]	94.33[a]	387.04[a]	494.18[a]	365.5[a]	378.05[a]
34	99.33[a]	95.33[a]	93.33[a]	95.33[a]	305.99[b]	487.29[a]	294.8[b]	304.39[b]
68	96.00[a]	89.33[b]	85.33[b]	88.33[b]	296.33[b]	326.24[b]	261.71[c]	239.21[c]
102	89.33[b]	88.66[b]	75.67[c]	77.00[c]	206.49[c]	261.69[c]	181.53[d]	185.86[d]
136	61.33[c]	79.33[c]	45.67[d]	47.33[d]	77.64[d]	148.06[d]	55.08[e]	54.04[e]
170	35.33[d]	49.33[d]	22.33[e]	25.00[e]	20.56[e]	40.77[e]	18.28[f]	20.55[f]
204	3.33[e]	14.00[e]	2.77[f]	4.54[f]	-	-	-	-

Values in a column followed by different letter(s) are significantly different at $p > 0.05$ of Duncan multiple range test. - represents seed not survived enough for measure.

Table 2. Effect of NaCl stress on seedling height and root length during early seedling growth.

NaCl (mM)	seedling height (cm)				Root length (cm)			
	YN	SF	SL	YD	YN	SF	SL	YD
0	2.37[b]	1.90[cd]	2.02[b]	1.92[b]	3.99[a]	5.01[a]	3.78[a]	4.14[a]
34	2.84[a]	2.67[a]	2.22[a]	2.07[a]	3.19[b]	4.20[b]	3.00[b]	3.40[b]
68	2.46[b]	2.29[b]	2.15[a]	1.98[a]	3.10[b]	3.66[c]	2.83[c]	3.19[c]
102	1.90[c]	2.01[c]	1.79[c]	1.56[c]	2.56[c]	3.22[c]	2.09[d]	1.99[d]
136	1.49[d]	1.84[d]	1.29[d]	1.39[d]	1.63[d]	2.67[d]	1.45[e]	1.49[e]
170	1.29[e]	1.43[e]	1.04[e]	1.05[e]	0.97[e]	1.55[e]	0.61[f]	0.72[f]
204	-	-	-	-	-	-	-	-

Values in a column followed by different letter(s) are significantly different at $p > 0.05$ of Duncan multiple range test. - represents seed not survived enough for measure.

and MDA contents in seedlings of YN, SF, SL and YD are shown in Figure 1. A gradual decline in POD activities of all species was marked at higher NaCl concentrations. The reduction of POD activity was strongest particularly at the highest levels of salt concentration compared to the control (Figure 1).

SOD activities of four species changed differently with the increase of NaCl concentrations, which were increased first at 34, 68 and 102 mM NaCl significantly and later decreased at 136 and 170 mM NaCl; although, these activities were lower as compared to the corresponding controls (Figure 1).

CAT activities of these four species showed a clear inhibition under salt stress as compared with the controls. CAT activities showed a reversed changing trend with SOD, which were decreased first at 34, 68 and 102 mM NaCl, and were later raised with increasing salinity from 136 mM to 170 mM NaCl (Figure 1).

MDA Contents of all species increased gradually with the increasing NaCl concentrations up to 170 mM NaCl (Figure 1). Higher levels of salt concentration caused MDA content accumulating more and more in plants during early seedling growth.

Effect of NaCl stress on contents of chlorophyll and carotenoid

Effect of salinity levels on chlorophyll and carotenoid contents in cotyledons of four cauliflowers is shown in Figure 2. Chlorophyll contents increased first and then decreased with increasing of salinity, and the highest chlorophyll contents of YN, SF, SL and YD were 37.35, 83.66, 81.52 and 70.85 $mg \cdot g^{-1}DW$ at 102 mM NaCl, respectively. The highest inhibition of chlorophyll contents were observed at 170 mM NaCl (Figure 2). Similar results were obtained in carotenoid contents in cotyledons of these four cauliflowers (Figure 2).

Effect of NaCl stress on root activity

Influence of salinity on root activities of four species is shown in Figure 3. There was considerable reduction of root activities in the species at all salinity levels. The reduction of root activity was strongest particularly at the highest level of salt concentration as compared to the control.

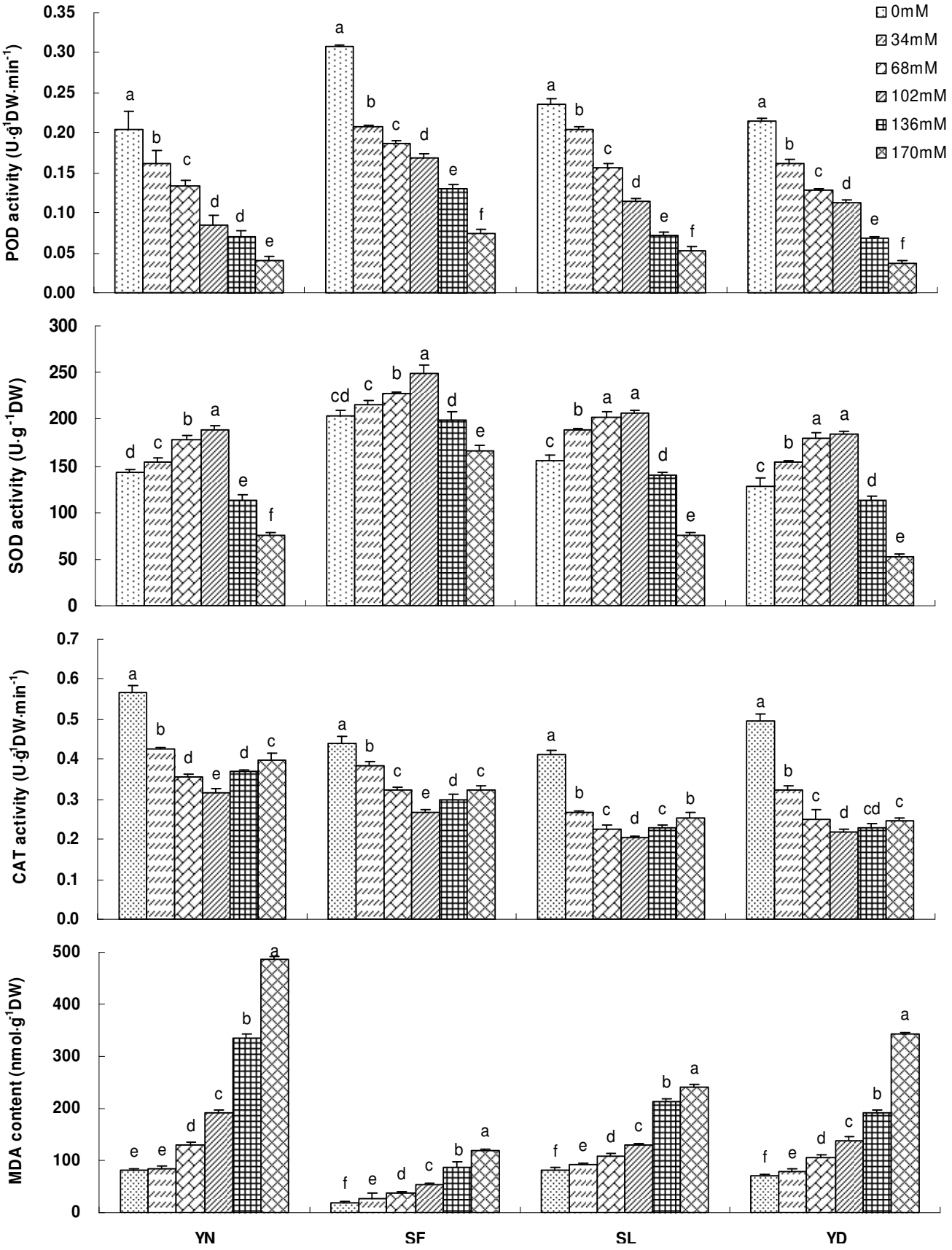

Figure 1. Effect of NaCl stress on POD, SOD, CAT activities and MDA content. The vertical bars represent standard errors.

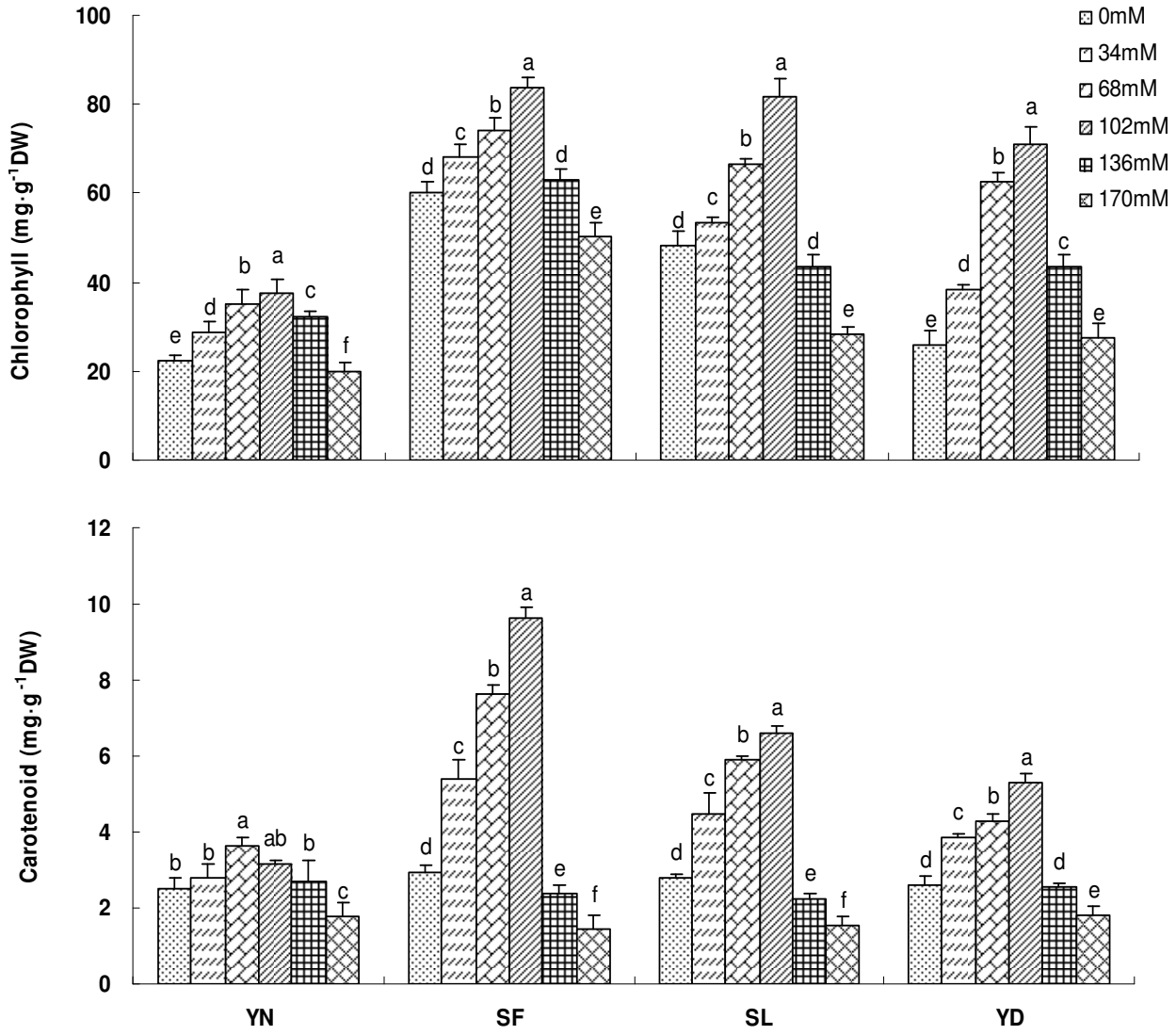

Figure 2. Effects of NaCl concentrations on contents of chlorophyll and carotenoid. The vertical bars represent standard errors.

Effect of NaCl stress on soluble protein content

Variations of soluble protein content of four species under different salinity are shown in Figure 3. Soluble protein contents were severely inhibited at all salinity treatments as compared to the control, and were decreased first at 34 and 68 mM NaCl and then increased at 102, 136 and 170 mM NaCl (Figure 3).

DISCUSSION

Germination stage is one of the most important phases in life cycle of plants and which is highly responsive to stress environment, such as salt, temperature, water, drought, etc. (Dash and Panda, 2001; Brini et al., 2009; Al-Taisan, 2010). This present study shows that germination percentage and vigor index of four cauliflowers was inhibited strongly particularly at highest levels of salt concentration (Table 1). Similar results were observed in other plant species (Dugan et al., 2004; Jamil et al., 2005, 2006, 2007; Al-Taisan, 2010; Dkhil and Denden, 2010). Extreme salt stress (204 mM NaCl) caused germination percentage decrease by more than 86%, while lower salt stress (34 mM NaCl) enhanced germination for YN, SL and YD more (Table 1). Similar result has been obtained in *Chenopodium quinoa* (Prado et al., 2000). It is assumed that extreme salinity might be toxic to the embryo of cauliflower seed and then the germination inhibited severely.Seedling height and root length were important parameters for salt stress because roots were in direct contact with soil, and absorb water from soil and seedling before supplying it to the rest of the plant (Jamil et al., 2006, 2007). We found that

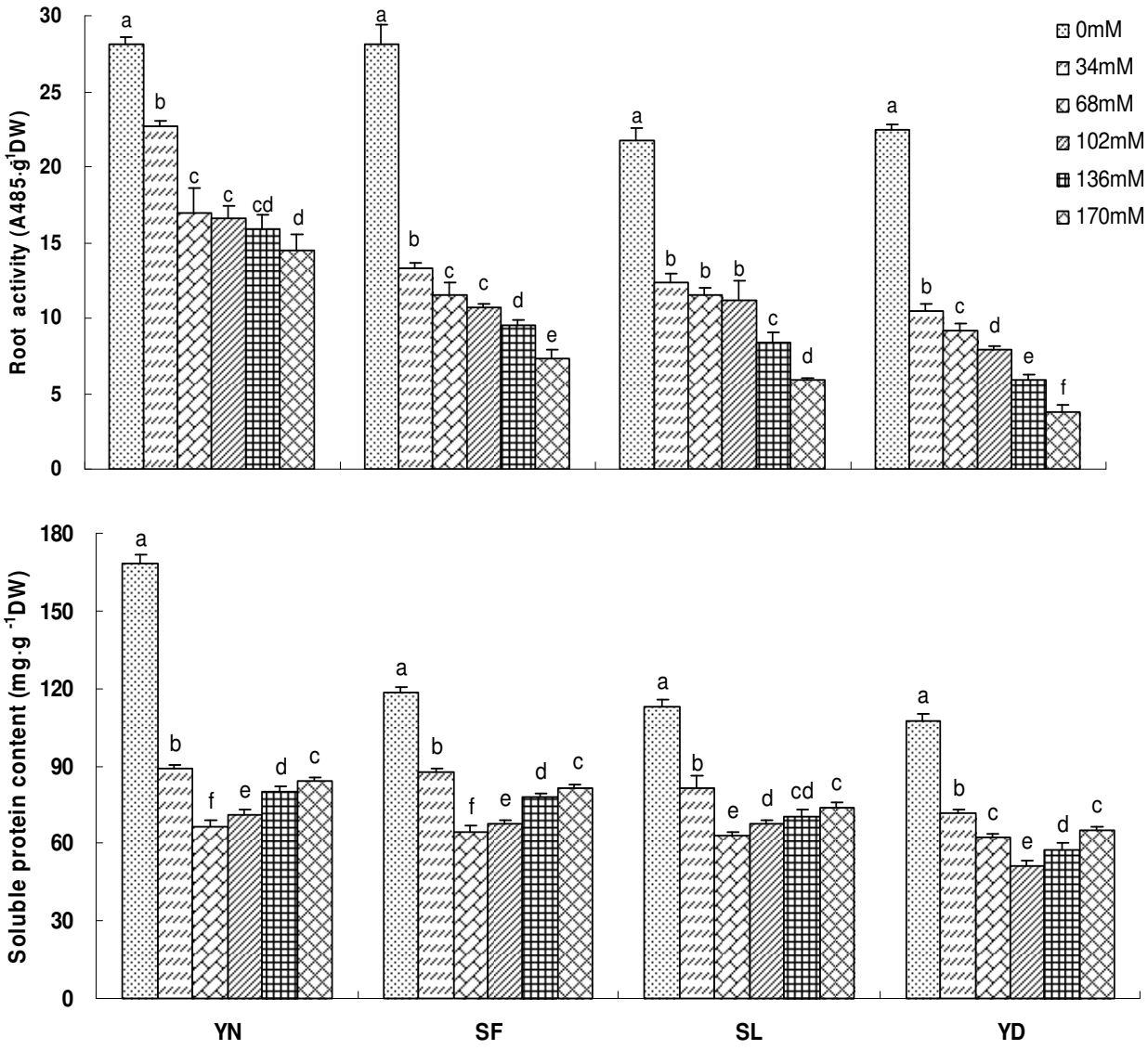

Figure 3. Effects of NaCl concentrations on root activity and soluble protein content. The vertical bars represent standard errors.

seedling height and root length during germination stage were inhibited severely especially at highest levels of salinity (Table 2). Similar result has been obtained in cauliflower and some other vegetables (Jamil et al., 2005, 2006, 2007). Inhibition of seedling growth by salinity might be due to the inhibitory effect of ions (Jamil et al., 2007; Brini et al., 2009; Souhail and Chaabane, 2009; Oueslati et al., 2010) or water absorption (Wener and Finkelstein, 1995; Prado et al., 2000). This might indicate that higher salt stress is not useful for seedling growth during germination stage. Salt stress induces reactive oxygen species (ROS) production and leads to oxidative damages. These toxic oxygen species may react with macromolecules and lipid components of membranes causing damage through lipid peroxidation resulting in increased permeability of the membrane (Singh, 2004). The antioxidant that protect system of plants comprises of a variety of antioxidant enzymes such as SOD, POD and CAT, etc., which were associated with scavenging reactive oxygen species (Oueslati et al., 2010). Antioxidant enzymes changed complicatedly and diversity during salt stress conditions, some studies reported that activities of SOD (Chinta, 2001; Diego et al., 2003; Asish et al., 2004), POD (Diego et al., 2003; Singh, 2004; Oueslati et al., 2010) and CAT (Chinta, 2001; Singh, 2004) increased, while others found that SOD (Azevedo Neto et al., 2004), POD (Saha and Gupta, 1997; Dash and panda, 2001) and CAT (Saha and Gupta, 1997; Dash and panda, 2001; Tijen and Ismail, 2005) decreased. Also someone found that SOD activity remained unchanged under salinity (Lechno et al., 1997). This present work shows that POD activities is inhibited

gradually with salinity, SOD activities increased first at lower salinity (34, 68 and102 mM NaCl) and then decreased at higher salinity (136 and 170 mM NaCl), while CAT activities changed reversely with SOD (Figure 1). It indicated that scavenging ROS mechanism of SOD, POD and CAT might vary in early cauliflower seedling at salt stress.

MDA is the decomposition product of polyunsaturated fatty acids of membranes under stress (Zhu et al., 2010a), content of which could be used as an indication to evaluate salt tolerance of plants indirectly (Azooz et al., 2009). We found that MDA contents in all cauliflowers were increased gradually with increasing NaCl concentrations up to 170 mM NaCl (Figure 2). Similar results were reported by Diego et al. (2003) and Tijen and Ismail (2005). It indicated that high salinity induced accumulation of MDA in seedlings which would be harmful for seedling growth and development.

Chlorophyll and carotenoid are important pigment for photosynthesis. We found that chlorophyll and carotenoid contents were increased at lower salt stress, while decreased at higher salt stress (Figure 2). Similar trends were observed in salt stressed maize (Sheng et al., 2008) seedlings. Root activities of the four species were strongly decreased by all salinity treatments (Figure 3). Similar result was obtained by Sheng et al. (2009), who reported that NaCl stress reduced root activity in maize plants.

Protein synthesis has been considered as a possible primary target of salt toxicity as reported by Gulen et al. (2006). Under salt stress, some studies reported that protein synthesis increased (Singh et al., 1987; Chandrashekar and Sandhyarani, 1995), while others decreased (Morant-Avice et al., 1998; Gulen et al., 2006; Oueslati et al., 2010) or remained unchanged (Singh et al., 1987). In this respect, we found that soluble protein content decreased at 34 and 68 mM NaCl and increased at 102, 136 and 170 mM NaCl (Figure 3). It is presumed that higher salt stress stimulated and increased soluble protein of seedling for salt tolerance.

Conclusion

Different salinity treatments had considerable effect on germination, early seedling growth, antioxidative enzymes activities, soluble protein and photosynthesis pigment contents during early seedling growth of cauliflower. Inhibition of germination percentage, vigor index and seedling growth was strongest particularly at highest levels of salt concentration. POD and root activities reduced gradually with salinity. SOD activities and chlorophyll and carotenoid contents increased first at 34, 68 and 102 mM NaCl and then decreased at 136 and 170 mM NaCl. Changing trend of CAT activity and soluble protein content was reversed with SOD etc. MDA contents increased significantly with increasing salinity up to 204 mM NaCl.

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