Interaction of Salinity and Cadmium on Physiological Response of *Brassica oleracea* L.

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Abstract

In order to survey effect of salinity and cadmium on catalase and peroxidase enzymes activity , proline accumulation, Malondialdehyde and protein content variation in *Brassica oleracea* an experiment was conducted in green house condition factorial design based on completely random block in 2014 year. The first factor was cadmium at four levels (0, 50, 100 and 200 ppm) and second factor was salinity at three levels (0, 50, 100 ppm). According to result of this experiment, catalase and peroxidase enzymes activity and proline and Malondialdehyde accumulation increased with Cd accumulation and protein content decreased. Under salinity stress peroxidase enzymes activity and Malondialdehyde accumulation increased. Interaction effect of cadmium and salinity cause to decrease Malondialdehyde accumulation, decrease protein content and decrease peroxidase and catalase enzyme activity.

Keywords: peroxidase, proline, catalase, cadmium, salinity ; Cauliflower .

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Introduction

Among the environmental stresses, salinity is a serious problem that about two million square kilometers of lands was used in agriculture that has affected by these cases. And therefore a major limiting factor in plants production around the world is considered. On the other hand increasing the salinity of the agricultural lands is widely expected, so that 30 percent of the lands in the next 25 years, and over 50 percent in 2050 due to the salinity of agricultural products withdrawn from stock (Wang et al., 2003). On the other hand, the earth as a salty planet is considered because most of the waters of it have about 30 gr of salt (sodium chloride) per liter. Water salinity was affected on the land which is farming in it. In around the world there are about 900 million hectares of lands affected by salinity and the development of it is considered a serious threat to the agricultural (Munns, 2000). Salinity in the soil or water is one of the main stresses in arid and semi-arid and can severely limit plant growth and production of plants (Koca et al., 2007; Allakhyediev et al., 2007). Hong and Redman in 1995 also mentioned the reduction of the chlorophyll amount in the leaves of barley plant in the salinity stress conditions. Na+ and Cl- ions are most commonly ions that were found in soil and salty water and both of them can have unfavorable effects on plants, because by

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increasing the osmotic pressure, the soil solution by creating ion toxicity in plant is disturbing the balance of plant is needed ions such as potassium (Hung & Redman 1995). The selection of salinity tolerant plants in all stages of plant life, especially at the germination stage is very important, In general uniformity in the emergence depends on the germination percent that these two kinds also are affected the salinity concentration amount and soil, water potential, nutrients, environmental temperature and interaction effect of these factors (Kaya, 2001). In addition to osmotic imbalances, ionic and nutritional in the plants, salinity also is disrupting the water absorption and biosynthesis of abscisic acid (ABA) in leaves that effect on the osmotic conductivity. This case effects on the photosynthetic electron displacement and enzyme activities (Parida & Das, 2005). From physiological and metabolic changes that may occur in response to salinity, ROS, such as Superoxide radicals (o2-) and Single Oxygen (o1-), hydroxyl radicals (oH-), and is accompanied by the formation of H2O2 (Mistra & Gupta, 2006). Plants have complex antioxidant system to avoid the harmful effects of ROS. The environmental stresses that cause increasing of ROS production cause oxidizing the photosynthetic pigments, membrane lipid, proteins and nucleic acids are (Smironoff, 1993). Plant cells to protection against oxidative damage are equipped with a broom of free radicals that among these cases can indicate to the catalase antioxidant, peroxidase and polyphenols peroxidase enzymes (Beltagi, 2008). Adaptation to the salt by increasing of the antioxidants amount to eliminate ROS is done supported by previous reports (Hernandez et al., 1993; Sehmer et al., 1995). The research showed that mycorrhiza fungus with extensive hyphal network and increasing the level and root rate absorption, the plants efficiency in the water absorption and nutrients, particularly sedentary elements such as phosphorus, zinc, copper will increase and cause the improving of them (Marschner, 1994). Studies have shown that the efficiency of microorganisms resistant to salinity can be effective in restoration and production of resistant materials (Rodriguez & redman, 2008). The existence of mycorrhiza fungus in the salinity soils and creating the symbiosis with the plants root in this circumstances indicate that probably some of these fungus is resistant to salinity stress and in the in symbiosis with plants by improving plant growth, increases the their tolerance to salinity (Yanamolo, 2003). The aim of this study was to investigate the effect of salinity on plant and antioxidant enzymes activities and also pretreatment mycorrhiza with salt was observed to investigate the resistance of mycorrhiza in the external environment.

Materials and Methods

This research in the greenhouse at the Islamic Azad University of Saveh branch was conducted. The experiment as a factorial form in a randomized complete block design with three replications was conducted. The first factor included mycorrhiza pretreatment in amounts of 0, 10, 50 and 100 mM salt and the second factor included the applying of salinity to the plant at the levels of 0, 25, 50, 100 and 200 mM. Prepared seeds of Seed and Plant Institute located in the city of Karaj by scientific name of (Hordeum vulgar) Reyhan cultivar before planting with five percent sodium hypochlorite disinfectant for 10 minutes and then sequentially washed with distilled water at three times and rinsed. Mycorrhiza Glomus fasciculatum fungi variety from the Research institute of Environmental Fanavaran Turan Shahrood was prepared and for each one kg pots from 40 grams fungus was used. To cultivate of pots with a height of 15 cm and a bottom diameter of 10 cm and a head diameter of 16 cm were used and 0.4 Leka mm to 10 cm of pots height was poured. After washing the seeds was soaked for 24 hours and also mycorrhiza in concentrations of 0, 25, 50, 100 mM sodium chloride salt (NaCl) for 24 hours salt pretreatment was placed. After the mycorrhiza time designated to remove salt residue washed and with swelled seeds of barley plant mixed and to the pots containing Leka at a depth of 2 to 3 cm were cultivated. In each plots 10 seeds were placed and was covered with a thin layer of Leka. Pots after preparing for 10 days with Hoagland made solution with a pH about 5.8 to 6 were irrigated daily and according to need of the plant. Pots in an environment with natural light and a temperature of 28 to 32 ° C were kept. To prepare the final solution in amounts of 0, 25, 50, 100 and 200 mM.lit-1 of sodium chloride salt was

used. Thus 7 liters Hoagland was prepared and in the and equal amounts were poured into 7 glass and sodium chloride salt at a concentration of 0, 25, 50, 100 and 200 mM.lit-1 was calculated and to the Hoagland solution into the glass was added then the treatment were applied on the pots for 10 days. For this reason, every day from the above treatment solution 2 times and each time in amount of 40 ml was added to each pot. After 10 days the applying of treatment, plants were harvested for experiments. First each plant slowly removed the leka so that the roots will not be damaged. Then root and small portion of the stem were washed with distilled water and additional water of it was taken. The organism of the plants fresh weight by using a Sartorius digital scale with 0.001 precision was calculated. After weighing the plant fresh weight at the aluminum foil in the oven at the 70 ° C temperature for 72 h were placed, then the plant dry weight was measured with a digital scale with a precision of 0.001. To obtain the examined plant leaf area, from the Canon 210 Lide model of canner device and leaf area meter software were used and its unit was measured according to the mm2. Chlorophyll measured by using of (Amon, 1949) method was conducted, to measuring the catalase activity from the (Cakmak & Horst, 1991) method, to measuring the peroxidase enzyme activity from the (Pandolfini et al., 1992), method, Polyphenols peroxidase enzyme activity from the (Kahn, 1975) method, for MDA from the (Valentovic et al., 2006) method and proline from the (Bates, 1973) method were used.

The obtained data from measurements with the Anova analysis by using the SPSS software was analyzed. Also the measured mean index by Duncan test at five percent probable was grouped.

Results

Plant fresh weight: based on the variance analyze results (Table 1) the effect of mycorrhiza pretreatment with salt and salinity treatment in plant at one percent probable and the interaction effect at the five percent probable has a significant effect on plant fresh weight. The obtained data from the mean comparison results of the plant fresh weight under the influence of mycorrhiza pretreatment factors with salt along with the salinity in the plant showed that the using of 25 mM salt in the plant in mycorrhiza pretreatment absence of plant fresh weight in comparing with control treatment increases but by increasing the salinity amount, plant fresh weight decreases. Also mycorrhiza pretreatment with salt and by applying the salinity in plant decreases the plant fresh weight. The mean comparison results of the plant fresh weight under the influence of mycorrhiza pretreatment with the different amount of salt in the presence of different levels of salt in the plant indicate that the most plant fresh weight in amount of 0.62 gr in the 25 mM salt treatment in the absence of mycorrhiza pretreatment and the lowest plant fresh weight in amount of 0.17 gr in the mycorrhiza pretreatment with 100 mM salt treatment along with using of 200 mM salt in the plant was obtained(Table 2).

Plant dry weight: based on the variance analyze results the effect of mycorrhiza pretreatment with salt and salinity treatment in plant at one percent probable and their interaction effect at the five percent probable has a significant effect on plant fresh weight (Table 1). Based on the obtained results from the mean comparison results of the plant dry weight under the influence of mycorrhiza pretreatment with salt salinity treatment in the plant, the plant dry weight by applying the 25 mM salt increases but by increasing the salinity, the plant dry weight decreases as well as mycorrhiza pretreatment with different amount of salt decreases the plant dry weight. So that the most plant dry weight in amount of 0.0474 gr is related to the 25 mM salinity in plant in the absence of mycorrhiza pretreatment and the lowest plant dry weight in amount of 0.0185 gr is related to the mycorrhiza pretreatment with 100 mM salt along with 200 mM salinity in plant was observed (Table 2).

Leaf area: based on the variance analyze results the effect of mycorrhiza pretreatment with salt, salinity treatment in plant at one percent probable and their interaction effect at the five percent probable has a significant effect on leaf area (Table 1). Mean comparison results of leaf area affected by the mycorrhiza pretreatment factor with different amounts of salt by applying salinity in plant (Table 2) suggest that mycorrhiza pretreatment with salt decreases the leaf area, also leaf area by applying the salinity to plant

Table 1
Analysis of variance of the effect of different levels of salinity and cadmium on the activity of catalase and peroxidase, proline and malondialdehyde accumulation and protein content variation in Cauliflower plant.

Variation source	Freedom degree	Catalase enzyme activity	Peroxidase enzyme activity	proline	Malondialdehyde	protein
block	2	1023209.82 ^{ns}	84756.06**	377.99 ^{ns}	9179.26*	1 ^{ns}
(a) cadmium	3	18039475.38*	665611.4**	5851.29**	30278.36**	4.32**
(b) salinity	2	6507337.23 ^{ns}	251385.04**	216.78 ^{ns}	14735.11**	0.12 ^{ns}
a*b	28	7659003.07 ^{ns}	133967.44**	5871.53**	798.69 ^{ns}	2.26**
erorr		4241945.4	9763.39	162.31	1838.6	0.36
CV%		23.26	13.14	27.05	15.19	23

** and * significant at one percent and five percent probability, respectively; ns: no significant effect

decreases. So that the most leaf area in amount of 14.94 mm2 is related to the control and the lowest leaf area in amount of 8.4 mm2 is related to the presence of 200 mM salinity in plant in the mycorrhiza pretreatment with 100 mM salt.

Peroxidase enzyme activity: based on the variance analyze results, the effect of mycorrhiza pretreatment with salt, salinity treatment in plant and their interaction effect at one percent probable on the peroxidase enzyme activity has a significant effect. The obtained data of mean comparison results of peroxidase enzyme activity influenced by mycorrhiza pretreatment factor with salt and salinity treatment to plant showed that with amount of salt as mycorrhiza pretreatment as well as salt levels in plant of peroxidase enzyme activity was increased so that the most peroxidase enzyme activity in amount of 880.33(Δ Abs mg-1 protein) is related to the 200 mM salinity in plant along with mycorrhiza pretreatment with 100 mM salt and the lowest peroxidase enzyme activity in amount of 103.53 (Δ Abs mg-1 protein) is related to the control treatment (Table 2).

Catalase enzyme activity: the obtained variance analyze results of the catalase enzyme activity measurement in the barley plant showed that mycorrhiza pretreatment with salt, salinity treatment in plant and also their interaction effect at one percent probable have significant effect on the catalase enzyme activity (Table 1). Based on the mean comparison results of catalase enzyme activity affected by mycorrhiza pretreatment factor with salt and salinity treatment in plant (Table 2) with mycorrhiza pretreatment by applying different amounts of salt and also salinity in the plant catalase enzyme activity in comparing with control increases so that the most catalase enzyme activity in amount of 133.38 (Δ Abs mg-1 protein) in the 200 mM salinity in plant with mycorrhiza pretreatment with 200 mM salt and lowest catalase enzyme activity in amount of(Δ Abs mg-1 protein) 11.18 in the control treatment was obtained that with 25 mM salinity treatment in the plant in the absence of mycorrhiza pretreatment with salt in amount of (Δ Abs mg-1 protein) 13.5 and also in the absence of salinity in the plant in the mycorrhiza pretreatment with 25 mM salt in amount of (Δ Abs mg-1 protein) 13.93 is placed in a statistical group.

Polyphenol oxidase enzyme activity: also variance analyzes results (Table 1) is evident mycorrhiza pretreatment with salt, salinity treatment in plant and also their interaction effect at one percent probable has significant effect on the polyphenol oxidase enzyme activity. Based on the obtained data of polyphenol oxidase enzyme activity mean comparison results affected by mycorrhiza pretreatment factor with salt and salinity treatment in plant (Table 2) with mycorrhiza pretreatment with salt different amount of polyphenol oxidase enzyme activity increases also by applying 200 mM salinity in the plant in the absence of mycorrhiza pretreatment

Table 2
Compared the effects of different levels of salinity and cadmium on the activity of catalase and peroxidase proline and malondialdehyde accumulation and protein content changes in Cauliflower plant.

cadmium (ppm)	salinity (ppm)	Catalase enzyme activity (U/g Plant)	Peroxidase enzyme activity (U/g Plant)	proline (µg/g DW)	Malondialdehyde (µ mol/g FW)	protein (mg/g FW)
0	0	4903. 55±761/01 ^{c}	353.38±52.63 ^{f}	7.91±3. 93 ^{d}	149.97±10.15 ^{d}	4.03±0.34 ^{a}
0	50	5939.09 ±244.76 ^{bc}	541.35±128.26 ^{ef}	9.38±3.52 d	191.66±14.81 ^{cd}	3.45±0.4 ^{abc}
0	100	9502.54 ±510.17 ^{ab}	541.35±65.11 ef	14.42±0.95^{d}	251.98±19.6 ^{bc}	2.47±0.47 ^{cde}
50	0	9380.71 ±496.81 ab	368.42±19.89 ^{f}	11.79±2.61 ^{d}	246.64±27.28 ^{bc}	2.11±0.43 ^{def}
50	50	10101.53±671.51 ^{a}	654.14±39.07 ^{e}	55.35±5.46 ^{c}	317.97±21.78 ^{ab}	3.2±0.34 ^{abcd}
50	100	7898.48±1429.24^{abc}	706.77±65.55 de	81.13±5.67 ^{ab}	309.97±20.82 ^{ab}	3.87±0.27 ^{ab}
100	0	9507.61± 514.87 ^{ab}	774.44±52.63 ^{cde}	82.78±13.88 ^{a}	286.64±32.83 ^{ab}	1.65±0.32 ^{ef}
100	50	9390.86±484.23 ^{ab}	917.29±92.39 ^{bcd}	81.9±5 ^{ab}	321.64±18.19 ^{ab}	2.21±0.38 ^{def}
100	100	10050.76±2067.55 ^{a}	969.92±65.11 ^{bc}	17.34±3.96 ^{d}	339.98±26.46 ^{ab}	2.74±0.44 ^{bcde}
200	0	8233.5 ±1020.85 ^{abc}	1097.74±52.63 ^{b}	87.59±5.34 ^{a}	293.32±49.78 ^{ab}	2.54±0.52 ^{cde}
200	50	11472.08 ±2112.55 ^{a}	646.62±99.46 ^{e}	57.89±18.21 ^{bc}	328.65±33.5 ^{ab}	2.01±0.24 ^{def}
200	100	9873.1±1405.6 ^{a}	1451.13±78.5 ^{a}	57.75±5.49 ^{bc}	349.98±41.46 ^{a}	1.02±0.16 ^{f}

Similar letters in each column showed no significant difference

with salt increases the polyphenol oxidase enzyme activity and statistically among other salinity levels has not observed the significant effect. The obtained results showed that by applying the salinity in plant in the presence of different amount of salt as mycorrhiza treatment of polyphenol oxidase enzyme activity increases so the most polyphenol oxidase enzyme activity in amount of (Δ Abs mg-1 protein) 1135.47 in the 200 mM salinity treatment in plant with mycorrhiza pretreatment with 200 mM salt is obtained.

MDA: based on the variance analyze results (Table 1, the effect of mycorrhiza pretreatment with salt, salinity treatment in plant and their interaction effect at one percent probable on the MDA has a significant effect. The obtained data of mean comparison results of MDA influenced by mycorrhiza pretreatment factor with salt and salinity treatment in plant showed that in the salt stress in the absence of mycorrhiza pretreatment, MDA in comparing with the control treatment increases. Also mycorrhiza pretreatment salt and by applying salinity stress in plant increases MDA. The MDA mean comparison results affected by mycorrhiza pretreatment with different amounts of salt in the presence of different levels of salt in plant indicate that the most MDA in amount of 14.03 nmol per gr of fresh weight in the 200 mM salt treatment in plant in the mycorrhiza pretreatment with 100 mM salt and the lowest MDA in amount of 5.18 nmol per gr of fresh weight in the control treatment was obtained.

Proline: variance analyze results showed that mycorrhiza pretreatment effect with salt, salinity treatment in plant and their interaction effect at one percent probable on the Proline has meaningful effect (Table 1). Based on the obtained results of mean comparison, Proline affected by mycorrhiza pretreatment with salt and salinity treatment in plant, by applying the salinity increases. Also the mycorrhiza pretreatment with

different amounts of salt increase Proline. The most Proline in amount of 27.09 µmol.gr Fw-1 is related to the 200 mM salt treatment in plant in the mycorrhiza pretreatment with 100 mM salt and the lowest Proline in amount of 3.44 and 2.77 µmol.gr Fw-1 are related to the control treatment and the absence of mycorrhiza pretreatment with salt in the presence of 25 mM salinity in plant (Table 2).

Discussion

Salinity by reducing the osmotic potential of soil makes difficult the water absorption by the plant (Prasad, 1997). Plants growing in salinity stress condition due to the reduction of water in root environment and specific effects of ions in the metabolic processes are reduced that the similar results by (Ghoulam et al., 2002) was obtained. Plants to have tolerant against salinity needs to osmotic adjustment and one of the osmotic adjustment methods is making organic material such as sorbitol, proline and glycine in the tissues. Making these materials to plants is associated with energy consumption. Therefore osmotic energy to osmotic adjustment reduces the growth and plant weight that with results by (Penuelas et al., 1997) are corresponded. Also the dry weight reduction of plant tissues due to increasing of metabolic cost and reduction of carbon by plant is to adapt to salinity (Netondo et al., 2004). Salt reduced the cells speed development and in high concentrations is stopped it. One of the plants adaptations to salinity is that the salt keeps in the out of their cells and this issue causes the water move out of leaf cells and the reduction of its levels. Sometimes leaf reduces the light absorption and dry matter production and reduces plant growth (Volkmar and Steppuha 1998), which it is in one direction with the results. Plant cells to protect against stresses damages, is equipped with a broom system of free radicals that one part from this system includes the antioxidant enzymes such as catalase and peroxidase (Cho et al., 2000). Catalases and peroxidases and polyphenol oxidases are very important enzymes that have an important stresses in response to abiotic stresses such as salinity stress. Catalase is one of the most H2O2 broom system that performs this act by converting the H2O2 to water and O2 (Dixit et al., 2001). Peroxidases are responsible for the removal of hydrogen peroxide amounts (Shalini & Duey, 2003). According to the obtained results in this study, by increasing of salinity stress amount at different levels of antioxidant enzymes treatment was increased. In this field the increasing of salinity will increase the activity of antioxidant enzymes, there are several reports is indicating that catalase, peroxidase and polyphenol oxidase enzymes activities increases (Sairam et al., 2001). Possibly the increasing of these enzymes activity in plants with increasing of stress shows that the studied plant from the antioxidant defense mechanism in order to resistance to stress is benefitted (Ajay et al., 2001). Stress can lead to changes in osmotic potential effect on a wide range of plants metabolic activities and with the formation of active oxygen radicals such as superoxides and hydrogen peroxide radicals leads to tension oxidative stress. Reactive oxygens are ion and osmotic severe stresses production that Leads to disruption of membrane structure and cell death (Bohnert & Jensen., 1996). Plants in confronting with these reactive oxygens, specific anti-oxidative enzymes such as catalase, peroxidase, polyphenol oxidase, and reductase glutathione and superoxide dismutase increase that with (RodrigueazRosales et al., 1999) results are corresponded. Researchers the increasing of malondialdehyde concentrations under salinity stress in corn (Gunes et al., 2007) and rice seedlings (Bandeoglu et al., 2004) reported that with the obtained results is corresponded. Destruction of cell membranes under salinity stress effect and the production of malondialdehyde that is resulting from the decomposition of cell membrane lipids can be investigated as a proper criterion to evaluate of tomato plants to salt stress. The increasing of Proline concentration in salinity stress condition may be due to the reduction of proline oxidation to glutamate or conversion of protein to proline that with the (Sannada et al., 1995) results are corresponded. Proline also provides the required energy for placement ions in the vacuole. In many halophytes plants, Proline or glycine betaine is enough to create an osmotic pressure that is involved in cells (Flower et al., 1977).

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