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Research Article

Effects of Mixed Salt Stress on Germination Percentage and Protection System of Oat Seedling

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Abstract: In order to determine the effect of mixed salt stress on the germination rate of naked oat and protective enzymes of its' seedling, in this study, using artificial culture box of climate, oat varieties (Yanke 1, Canadian 9, Big oat, Canadian 8, Canadian 6 and Baoluo) were cultivated in mixed salts solution. The results showed, with the increasing of concentration of double salts, the germination ratio and plant height decreased, semi-lethal concentration was between 1.5% and 2.6, utmost concentration was between 2.3 and 3.8%, respectively; the CAT activity of Big oat decreased, but that of other varieties increased first and then decreased, the peak value appeared when concentration of double salts was between 0.4 and 0.6%, respectively. And with the increasing of the concentration of double salt, POD activity and SOD activity of six oat varieties increased first and then decreased and its highest value appeared when concentration of double salts was between 1.2 and 1.8% respectively; the proline contents of all varieties increased, while the MDA contents decreased first and then increased and its minimum value appeared when concentration of double salts was 0.2%. To sum up, under the condition of salt stress, it was likely because of increasing of protective enzyme activity and proline contents to make salt tolerance of oats higher and salt tolerance of different varieties showed significant difference. The salt tolerance order showed that Yanke 1>Canadian 9>Baoluo>Big oat>Canadian 8>Canadian 6.

Keywords: Malondialdehyde, mixed salt, oat, proline, protective enzyme system, seedling

INTRODUCTION

Currently, the area of saline land has reached 950 million ha in the world and in China, the area is 20 million ha which is 10% of the total cultivated area. The soil salinization has been an important environmental problem (Zhao *et al*., 2003; Zhang *et al*., 2005). With the increasing of fertilizer amounts, soil secondary salinization is becoming more and more serious, the salt stress almost affects the whole growth period of plant. And in the saline environment, plant can't prevent the salinity entering the body, it only can take different physiological measures to adjust the salinity, or to exclude the salinity, then to keep the normal physiological activities (Shi and Bao, 2007; Huang *et al*., 2008). So the crop growth under salt stress, the physiological and ecological response mechanism of salt resistance and salt tolerance have been the important research subjects in the agriculture and ecology domain (Ding *et al*., 2001; Qiu *et al*., 2006). In the soil, NaCl, Na_2SO_4, Na_2CO_3, $NaHCO_3$ and $MgCl_2$ are the main salinity which causes saline-alkali stress. Oat is the main crop which is used to control the saline land, so in this study, we are making the attention on the effects of double salt stress on oat growth and protective enzymes activity.

Through the synergistic effect of anti-oxygen protective enzyme system including SOD, CAT, POD and the other system including AsA, GSH, active oxygen can be produced and eliminated continuously and then to protect the normal metabolism level of plant from being damaged (Tang *et al*., 2004; Tian *et al*., 2005). Recent years, people have studied much about the active oxygen produced by environment stress and the damage of it on the growth of plant. It is showed that under serious stress, the activity of cell protection system which the SOD is main decreases, causing the change of protoplasm membrane lipids and membrane structure, the increasing of relative conductivity and MDA (Dhindsa, 1987; Jiang *et al*., 1991; Cao *et al*., 2004; Sun *et al*., 2004). Under mild stress, the increasing of O^2 and H_2O_2 can induce the increasing of protective enzyme activity such as SOD, POD and CAT and increase their resistance (Jiang *et al*., 1991, 1994; Zhu and Huang, 1994; Tang and Li, 1995; Chen and Zhang, 2000). Proline is the amino acid which has the

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biggest water-soluble, under salt stress, the synthesis of protein is inhibited and the decomposition is promoted, at last the content of amino acid increases (Wang *et al*., 2003), so under different environmental stress, plant can use the change of different enzymes to adjust the normal physiological characters, increase the tolerance. The change of enzymes under stress has important effects on the growth of plant. Some researchers think that in the seedling stage and jointing to booting stage, the higher soil salinity can cause salt damage (Ding *et al*., 2001; Zhao and Dou, 1998). In order to fill the gap of salt tolerance of oat, this study simulating the micro environment of saline land, choosing 6 oat varieties as experimental materials, through the analysis of the change law of germination rate and protective enzyme activity to definite the effects of different mixed salt stress degree on the growth and physiology and ecology of oat seedling, making clear the different resistance and tolerance of different oat varieties and then providing the theoretical foundation for the controlling of saline land, providing theoretical basis for making clear the salt tolerance mechanism of oat.

MATERIALS AND METHODS

Experimental materials: Choose the varieties which the germination percentage is higher than 85%, Including Yanke 1, Canadian 9, Baoluo, Big oat, Canadian 8 and Canadian 6. The tested salinities and their proportion are as follows: the content of NaCl is 27.6 g, Na_2SO_4 is 27.46 g, $MgSO_4$ is 5.94 g, $CaCl_2$ is 2.727 g, $NaHCO_3$ is 0.985 g and Na_2CO_3 is 0.015 g. And dissolving these 64.73 g into distilled water made it as mixed salt solution. And then from the lowest salt concentration is 0.0%, increased by 0.2%, to the highest is 3.8% and totally include 20 salt gradients.

Experimental methods: In the laboratory, taking the methods of germination on the study plates and choosing 30 ripe, plump and uniform seeds. First soaking them by 0.1% $HgCl_2$ for 8 min, then soaking them by 10 mL the different salt solution (the control using distilled water) for 12 h, then fully washing them by distilled water and sipping up the water of seeds by filter paper. Then put them in the culture dishes which covered by two layers filter paper and have 10 mL corresponding salt solution in them, repeating for three times. Then putting the culture dishes into the artificial climate incubator (SPX-250 IC), under the condition of temperature is 25°C thermostatic, humidity is 80% and light interval is 12 h culturing for 15 days. After 15 days, determining the indexes of seedling. And from the second day in the artificial climate incubator, observing and recording the germination number daily and continuously for 7 days. In this progress, the water of evaporation should be supplied by distilled water and make the water of filter paper keep in the saturation state.

Testing indexes and the methods: In the seventh day of mixed salt stress, the germination rate was determined. Germination rate = germination number/total seed number *100%. In the fifteenth day of mixed salt stress, the plant height of seedling was determined; each repeat of one treatment was determined 5 plants and total 15 plants. Then taking the leaves of seedling to determine the physiological index, the determination was repeated for 3 times. Each physiological index was determined until the gradient after median lethal concentration. SOD was determined by NBT light oxidation reduction method (Li, 2000), POD was determined by guaiacol method (Li, 2000), CAT was determined by Cak-mak method (Cakmak and Marschner, 1992), Pro was determined by sulfosalicylic acid method (Yang *et al*., 2006), MDA was determined by TBA method. The median lethal concentration is the mixed salt concentration which the germination rate is 50% of the control. The limiting concentration is the mixed salt concentration which the germination rate is 10% of the control (Qi *et al*., 2001).

Data statistics: The variance analysis was done by the DPS3.0 and SAS9.0.

RESULTS AND ANALYSIS

Effects of mixed salt stress on the germination rate of different oat variety. From Fig. 1, the total change trend of germination rate of 6 oat varieties all showed: with the increasing of the concentration of mixed salt, the germination rate decreased, but except the concentration of 0.2%. When in the concentration of 0.2%, the germination rates of Big oat and Canadian 6 both were higher than the control, increased by 7.44 and 5.52%, but the germination rate of Yanke 1 was equal to the control, the germination rates of Canadian 8, Canadian 9 and Baoluo were lower than the control, decreased by 7.07, 2.86 and 3.52%, respectively. With the increasing of concentration of mixed salt, the change of germination rate of Yanke 1 and Canadian 9 which had the biggest limiting concentration showed gentler, it of Canadian 6, Canadian 8 and Baoluo showed more violent and it of big oat showed the most violent, showed a linear decrease trend after the median lethal concentration. And with the increasing of concentration of mixed salt, the germination of 6 oat varieties all showed a hysteresis effect and mainly

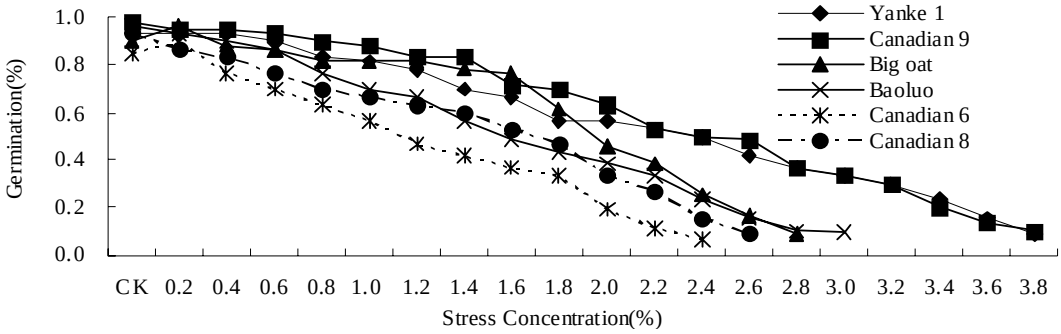

Fig. 1: The change of germination rate of oat under mixed salt stress

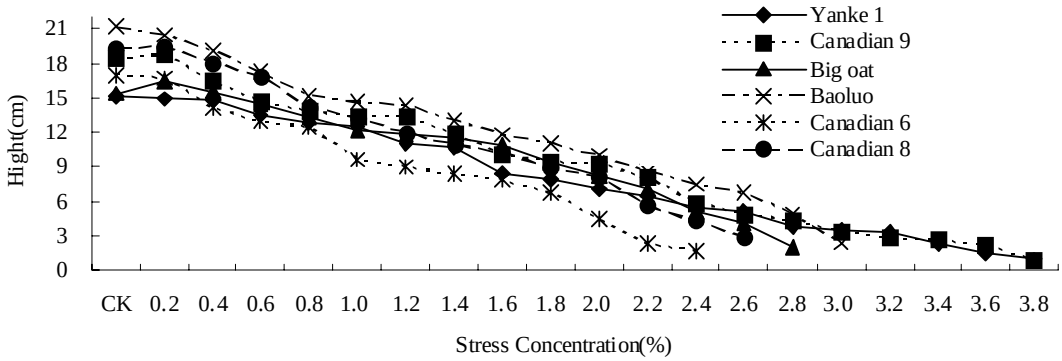

Fig. 2: The change of plant height of oat under mixed salt stress

showed the decreasing of germination rate and prolonging the time of germination needed. Through the significance analysis, when the concentration of mixed salt was 0.2%, the germination rate of each variety had no significant difference with the control and under other concentration; the germination rate of each variety all had significant or very significant difference with the control.

Effects of mixed salt stress on the plant height of different oat varieties: When the plant was damaged by salt, it always shows dwarf, thin and weak, reduction in tiller number, thin leave, leaves with a pale yellow color, with the time prolonging, showed water loss and wilting of leaves, when it is serious it can be die. From Fig. 2, with the increasing of concentration of mixed salt and the time of stress, the damage degree all increased, but there were difference among treatments. Under the concentration of 0.2%, the plant height of Canadian 9, Canadian 8 and big oat increased by 2.16, 1.04 and 7.04%, respectively than the control, except for this, under other stress concentration, the height of each variety all lower than the control. At the median lethal concentration, the height was only 50% of the control and at the limiting concentration, the height of Yanke 1, Canadian 9, Canadian 8, Canadian 6, Baoluo and Big oat were 1.0, 0.9, 2.9, 1.7, 2.4 and 2.1 cm, respectively. Through the significant analysis, when the concentration of mixed salt was more than 0.4%, the height of each oat variety all had very significant difference ($p<0.01$).

Effects of mixed salt stress on the median lethal concentration and limiting concentration of different oat varieties: From Fig. 3, Canadian 9 had the biggest median lethal concentration of 2.6%, second was Yanke 1, Canadian 6 had the lowest median lethal concentration of 1.5%. Canadian 9 and Yanke 1 had the biggest limiting concentration of 3.8%, second were Baoluo, Big oat, Canadian 8 and Canadian 6, their concentration were 3.0, 2.8, 2.6 and 2.3%, respectively. The median lethal concentration and limiting concentration between Canadian 9 and Yanke 1 had no significant difference, them among other oat varieties all had significant difference ($p<0.05$) and there had very significant difference between Canadian 9 and Yanke 1, among Big oat, Baoluo and Canadian 8 in the median lethal concentration and limiting concentration, the median lethal concentration and limiting concentration of Canadian 9, Baoluo, Big oat, Canadian 8 and Yanke 1 had very significant difference ($p<0.01$) with Canadian 6.

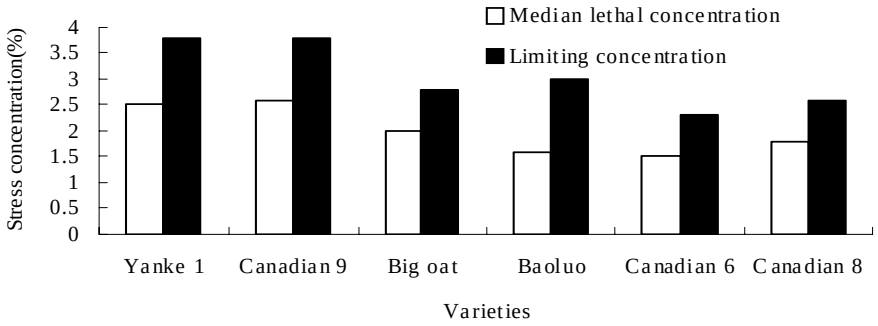

Fig. 3: The change of median lethal concentration and limiting concentration of different oat varieties under mixed salt stress

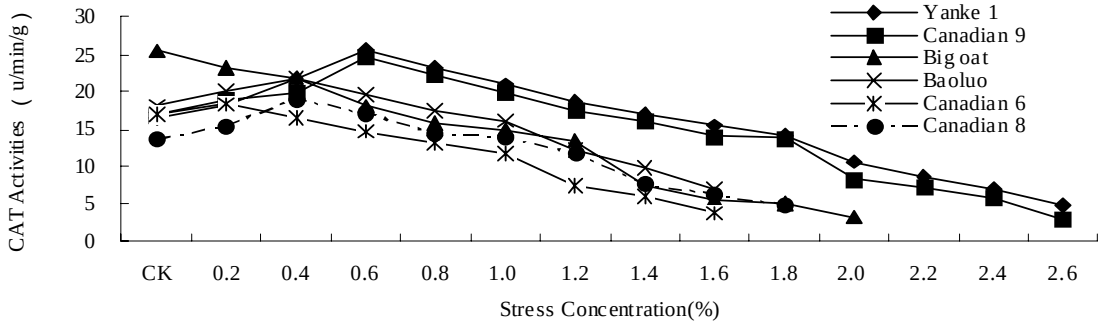

Fig. 4: The change of CAT activity of seedling leaves of different oat varieties under mixed salt stress

Effects of mixed salt stress on CAT activity of different oat variety: From Fig. 4, with the increasing of concentration of mixed salt, the CAT activity of big oat decreased, but it of the Yanke 1, Canadian 9, Baoluo, Canadian 8 and Canadian 6 all showed a first increased and then decreased trend. When they reached the biggest activity, the concentration of mixed salt of them were 0.6, 0.6, 0.4, 0.4 and 0.2%, respectively. The biggest activity of them was 1.55, 1.46, 1.21, 1.38 and 1.08 times of that of the control, respectively. When the concentration of mixed salt was 0.6%, the CAT activity of big oat was 70.7% of that of the control, and under the each stress concentration, the CAT activity of Big oat all had significant ($p<0.05$) or very significant difference ($p<0.01$) with the control. The decreasing degree of CAT activity of Yanke 1 and Canadian 9 were gentle compared with other varieties and CAT activity of Canadian 6 was only higher than Canadian 8 under concentration of 0.2%, under other treatments it were all lower than other varieties. Through significant analysis, the CAT activity of different oat varieties under different concentration of mixed salt all had significant ($p<0.05$) or very significant ($p<0.01$) difference.

Effects of mixed salt stress on POD activity of seedling of different oat variety: From Fig. 5, with the increasing of concentration of mixed salt, the change of POD activity of different oat variety all showed a single peak curve with first increasing and then decreasing. But there existed difference in the concentration of mixed salt which the POD activity of each oat variety reached the biggest value. Under the concentration of 1.8%, Yanke 1 and Canadian 9 reached the biggest POD activity, under the concentration of 1.4%, big oat; Baoluo, Canadian 8 and Canadian 6 reached the biggest POD activity. And between the concentration of 0.2 to 1.6%, POD activity of big oat was lower than that of the five varieties. Through significant analysis, under the concentration of 0.2%, the POD activity of Yanke 1 and Canadian 9 had no significant difference with the control. Under the other concentration, the POD activity of different oat variety all had significant ($p<0.05$) or very significant ($p<0.01$) difference with the control.

Effects of mixed salt stress on the SOD activity of seedling of different oat variety: SOD is a key enzyme in oxygen metabolism inside plant, it can catalytic the disproportionation, the activity of it can affect the content of O_2 and H_2O_2 inside plant. From Fig. 6, with the increasing of concentration of mixed

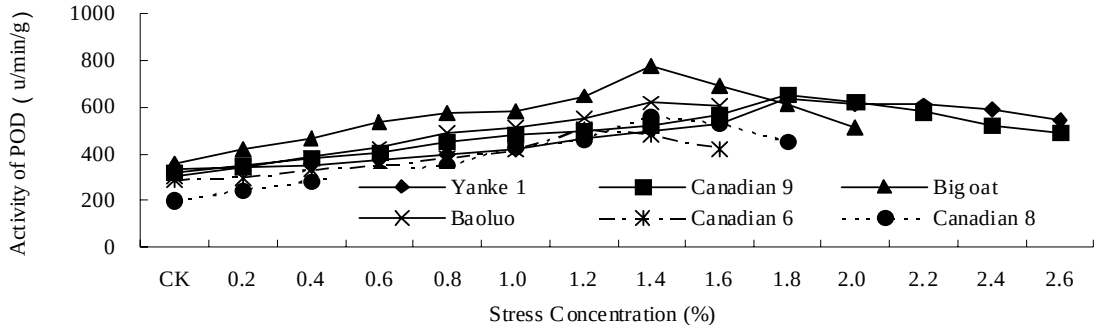

Fig. 5: The change of POD activity of seedling leaves of different oat variety under mixed salt stress

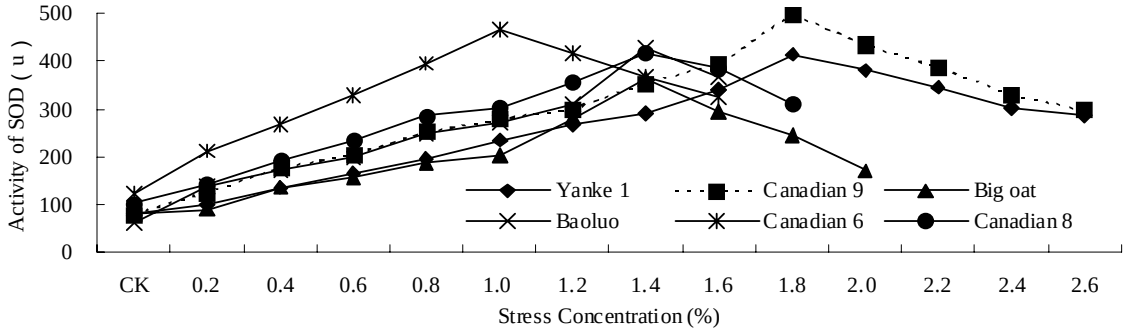

Fig. 6: The change of SOD activity of oat seedling leave under mixed salt stress

salt, the SOD activity showed a single peak curve with first increasing and then decreasing. This indicated that low salt concentration can cause the increasing of the SOD activity in oat leave; it can clear free radical and prevent the peroxidation and damage of membrane. When the concentration was over a certain range, the SOD activity decreased and this indicated that exorbitant concentration can inhibit the SOD activity. Under the concentration of 1.8%, the SOD activity of Canadian 9 and Yanke 1 reached the biggest value of 497.4 and 413.7 u/g, respectively. Under the concentration of 1.4%, the SOD activity of big oat, Baoluo and Canadian 8 reached the biggest value of 365.4, 429.9 and 418.9 u/g, respectively. And under the concentration of 1.0%, the SOD activity of Canadian 6 reached the biggest value of 467.2 u/g.

The Canadian 6 had the lowest limiting concentration, but the SOD activity of it was higher than that of the other varieties under each treatment. Under mixed salt stress, SOD activity of different oat variety showed an opposite "V" model; this can indicate that the SOD activity had relationship with the salt tolerance of oat seedling, so SOD activity can be used to identify the salt tolerance of oat.

Effects of mixed salt stress on the proline content of seedling of different oat variety: From Fig. 7, with the increasing of concentration of mixed salt, the proline content of 6 oat varieties increased. Under the concentration of 0.2%, the proline content of Yanke1, Canadian 9, Big oat, Baoluo, Canadian 6 and Canadian 8 were 245.1, 105.4, 84.85, 109.4, 128.2 and 106.0 ug/mg, respectively compared with the control, they were 1.04, 1.19, 2.08, 1.63, 1.14 and 1.15 times, of the control respectively. Under the median lethal concentration, the proline content of 6 oat varieties were 2.87, 6.35, 8.83, 6.10, 3.54 and 4.65 times, respectively of the control, the increasing was obviously. This indicated that proline was the sensitive index when plant was under stress. Through significant analysis, the proline content of each variety and each treatment all had significant or very significant differences.

Effects of mixed salt stress on the MDA content of seedling of different oat variety. From Fig. 8, with the increasing of concentration of mixed salt, the MDA content of 6 varieties showed a change trend of decreased first and increased then, the lowest value appeared when the concentration of mixed salt was 0.2%. Under the concentration of 0.2%, the MDA content of Yanke 1, Canadian 9, big oat, Baoluo, Canadian 6 and 8 were 3.87, 2.79, 4.26, 3.91, 3.43 and 2.26 mmol/mg, which were the 89.7, 90.0, 99.2, 94.5, 88.6 and 98.6%, of the control, respectively. Under the

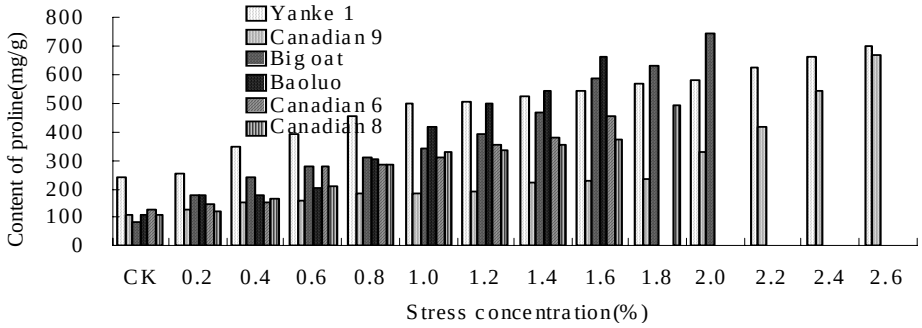

Fig. 7: The change of proline content of oat seedling under salt stress

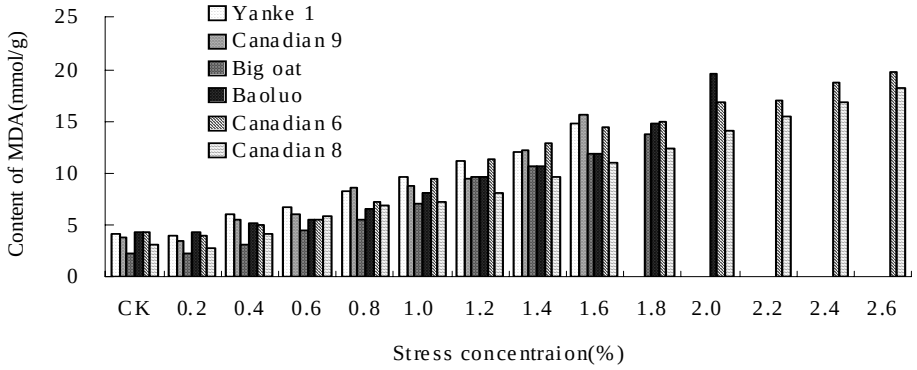

Fig. 8: The effect of mixed salt stress on MDA content of oat seedling

concentration of 1.4%, the MDA content of 6 varieties were 2.97, 3.12, 2.49, 2.93, 3.16 and 4.66 times of the control respectively. And from the figure, the concentration of 0.2 and 1.4% were the turning point of MDA content. When the concentration was 0 to 0.2%, the MDA content of 6 varieties all decreased, this was because that 0.2% salt content can induce the increasing of protective enzyme activities and also can clear the active oxygen effectively and then decreased the MDA content. When the concentration was 0.2 to 1.4%, the MDA content of 6 varieties increased slowly, when the concentration was 1.4 to 2.2%, the MDA content increased acutely, this was because under these concentration, the activity of protective enzyme began to decrease with a high speed, the active oxygen was accumulated, the peroxidation was serious that cause a large amount of MDA. Through significant analysis, under concentration of 0.2%, the MDA content of each variety had no significant difference and there were significant or very significant differences among each variety under each treatment.

CONCLUSION AND DISCUSSION

Effects of mixed salt stress on seed germination and seedling height: With the increasing of concentration of mixed salt, the germination rate showed significant difference in different variety. This study showed, with the increasing of concentration of mixed salt, the germination rate decreased. Under the concentration of 0.2%, the promoting and inhibitory effect of salt stress on the germination of 6 varieties was small. When the concentration was more than 1.0%, the inhibitory effect was bigger. Foolad and Lin (1999) thought under the stress conditions, the germination speed of seed showed invariable and there maybe existed stress tolerance gene or special physiological mechanism, so this was similar with that the effect of low concentration stress on germination was small. In this study, the change trend of plant height was similar with the germination rate, both showed under the concentration of 0.2%, the plant height of oat was higher than the control and under other concentrations; the plant height was lower than the control. This was because that many salt ions accumulated on the oat root, this prevented the absorption of water and other useful ions and then the synthesis and accumulation of organic matter was inhibited, so the slow growth caused the lower plant height.

Effects of mixed salt stress on protective enzyme activity: When the plant was under stress, the dynamic

balance of the production and clearance of active oxygen was damaged, each protective enzyme activity was inhibited. In this study, the CAT activity of big oat decreased with the increasing of concentration of mixed salt. The CAT, SOD and POD activities of Yanke 1, Canadian 9, Big oat, Baoluo, Canadian 6 and Canadian 8 were all showed increased first and then decreased with the increasing of concentration of mixed salt. Under the concentration of 0.6%, the CAT activity of Yanke 1 and Canadian 9 reached the biggest, under the concentration of 0.4%, the CAT activity of Canadian 8, Canadian 6 and Baoluo reached the biggest. When the SOD and POD activities of Big oat and Baoluo reached the biggest, the concentration of mixed salt was 1.4%, when the SOD and POD activities of Yanke 1 and Canadian 9 reached the biggest, the concentration of mixed salt was 1.8%, when the SOD and POD activities of Canadian 6 reached the biggest, the concentration of mixed salt was 1.2%. This indicated that when the concentration of mixed salt was lower than 1.4%, the naked oat variety can maintain the membrane system and it had some salt tolerance, so under low mixed salt concentration, the ability of oat seedling to clear active oxygen increased which was helpful to maintain the stable of membrane of oat seedling and was also helpful to prevent the entering of germ. But under the high mixed salt concentration, the activity of protective enzyme decreased, this indicated that the regulating capacity of protective enzyme was limited which was matched with the results of Ren *et al*. (1997).

Effects of mixed salt stress on the content of proline and MDA of oat seedling: When the plant was under the stress environment, the unsaturated fatty acid in plasma membrane would occurred peroxidation and then produce MDA and cause the damage on membrane system, selective permeability was damaged and the electrolyte leakage would increase, so the MDA content can reflect the strength of the peroxidation of membrane (Sun *et al*., 2001). In the results of this study, under the mixed salt stress, when the concentration was 0.2%, the MDA content of oat seedling decreased, but except the concentration of 0.2%, the MDA content increased under the other concentrations. This indicated that mixed salt stress made some damages on cell membrane of oat seedling. In this study, with the increasing of concentration of mixed salt, the proline content increased, this may because that under stress environment, the synthesis of protein was inhibited and the amino acid content increased, especially the synthesis of proline was activated obviously (Friedman and Altman, 1989), speed up the synthesis of proline and increased the solute osmotic potential of plant cells, the internal and external infiltration intensity of protoplast were balanced, maintained the normal structure of enzyme in cells, decreased the precipitation of soluble protein in cells, increased the resistance of plant and protected plant from being damaged.

Analysis on the salt tolerance of 6 oat varieties under mixed salt stress. The plant salt tolerance is a complex physiological progress; each single mechanism research can't evaluate the plant salt tolerance completely (Hou *et al*., 2005). In this study, there were big differences in the salt tolerance of 6 oat varieties, such as the median lethal concentration of Yanke 1 and Canadian 9 were higher, but the protective enzyme activity of them were lower. The protective enzyme activity of Baoluo and Big oat were higher, but the median lethal concentration and limiting concentration of them were lower than that of Yanke 1 and Canadian 9. The median lethal concentration, limiting concentration and protective enzyme activity were all lower, this may because that Canadian 9 and Yanke 1 used the physiological change to resist the salt stress and they had the salt tolerance gene, Baoluo and big oat mainly used the physiological change to resist the salt stress.

In a word, when the concentration of 0.2% had promoting effects on the germination of oat seed and the growth of oat seedling, 0.2 to 0.6% was the concentration which the 6 oat varieties were under low stress, under these concentrations, the protective enzyme activity and the proline content all increased which decreased the damages of salt stress on oat. (1.0 to 1.4%) was the concentration which the oat varieties were under moderate stress, although the protective enzyme activity also increased, but the speed of accumulation was higher than that of the clearance, causing the accumulation of active oxygen, the membrane was damaged and accumulating a large amount of MDA. More than 1.6% was the concentration which the oat varieties were under serious stress, the protective enzyme activity decreased rapidly, the content of proline and MDA increased rapidly, the physiological mechanism were damaged seriously and the physiological metabolism can't normal proceeding, so the oat seedling growth were inhibited, this was reflected by the plant height of 6 oat varieties. According the analysis of median lethal concentration, limiting concentration and each physiological indexes, under the certain salt stress, maybe because the increasing of protective enzyme activity and proline content, the oat salt tolerance increased, also maybe that oat itself had the salt tolerance gene and there were differences of salt tolerance among different oat varieties and the further research of analysis on gene diversity and the calibration of oat salt tolerance gene would explain this question.

Under salt stress, the germination and plant height of Canadian 6 were inhibited seriously and the protective enzyme activities were all low, it was the variety with low salt tolerance. The median lethal concentration, limiting concentration, plant height and germination were all high; they were the varieties with high salt tolerance. The germination, median lethal concentration and limiting concentration of Canadian 8, Baoluo and Big oat were among the above 4 varieties, they were the varieties with medium salt tolerance. Through the comparison of median lethal concentration, limiting concentration, germination, plant height and each physiological indexes, the order of salt tolerance of 6 oat varieties showed Yanke 1>Canadian 9>Baoluo>Big oat>Canadian 8>Canadian 6 in the seedling stage.

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