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AMELIORATION OF SODIUM CHLORIDE STRESS IN *VIGNA UNGUICULATA* (L.) WALP.

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ABSTRACT

Petri dish and pot experiments were conducted for amelioration of sodium chloride stress in *Vigna unguiculata*. Petri dish experiments included three sets (25 mM, 50 mM, 75 mM, 100 mM, 125mM sodium chloride – 10ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm ammonium molybdate – 25 mM + 10 ppm, 50 mM + 10 ppm, 75 mM + 10 ppm, 100 mM + 10 ppm, 125 mM + 10 ppm sodium chloride and ammonium molybdate). Control, treated with distilled water, was also maintained. Seed germination was 100 percent in control, 10 ppm ammonium molybdate, 25 mM + 10 ppm and 50 mM + 10 ppm treatments only. An increase in germination percentage of seeds treated with combination of sodium chloride and ammonium molybdate, over the treatments of different concentrations of only sodium chloride, was recorded. Fifteen-day old seedlings, grown in pots with similar treatments, were used for the analysis of length, dry biomass, chlorophyll, starch and total free amino acids. All the parameters studied were found to be more in the seedlings treated with 50 mM + 10 ppm solution than those treated with different concentrations of sodium chloride (50 mM, 75 mM, 100 mM) and combinations of higher concentrations of sodium chloride and ammonium molybdate (75 mM + 10 ppm, 100 mM + 10 ppm). The values of 50 mM treatment were nearing those of control. This must be due to the ameliorating effect of ammonium molybdate on sodium chloride stress. The present work suggests the use of lower concentration of ammonium molybdate in ameliorating negative effects of sodium chloride stress in *Vigna unguiculata*.

INTRODUCTION

Soil salinization is one of the major factors caused by human beings by creating irrigation infrastructure. About 10,000 hectare of land is being added every year to the existing 9.8 million hectare of saline soil in our country (Singh et al. 1999). The saline soils have a pH of less than 8.5, exchangeable sodium less than 15 percent and excess of chloride and sulphate of sodium, calcium and magnesium. Such soils are low in fertility, and plant growth in them is impaired mainly by osmotic effects of excess salts and nutritional imbalance. The absorption of water and minerals by plants is affected by disturbed equilibrium. Poor seed germination, stunted growth, disturbed physiological activities, delayed flowering and reduced yield are the responses of plants growing in salt affected soil.

Poor seed germination and growth of different cultivars of rice due to salt stress was reported by Subburamu & Ramamoorthy (2003). Reduced yield of ragi (Deepak Bhat & Hosmani 1991) and wheat (Sanwal & Varshney 1997), low chlorophyll content in rice (Salunkhe & Karadge 1989), less glutamate dehydrogenase and nitrate reductase activities in pigeon pea (Amrit Sagar & Vaidyanath 1993) were also reported due to salinity.

Various physical, chemical and biological methods have been suggested to overcome salt stress, which include scraping, flushing, leaching, application of chemicals, selection of salt tolerant crops and improving genetic resistance of crops. All the applications of chemicals have been found to be successful. The use of gibberellic acid (Deepak Bhat & Hosmani 1991), 6-benzyl aminopurine (Amrit Sagar & Vaidyanath 1993), Ca^{++} ions (Sahoo & Sahu 1994), triadimefon (Muthukumarasamy &

Panneerselvam 1997), molasses and gypsum (Sanwal & Varshney 1997), boron (Mashad & Kamel 2001), calcium nitrate and potassium nitrate (Cengiz Kaya et al. 2003) and ammonium sulphate (Khajuria & Singh 2006) have been reported in ameliorating salt stress. Perusal of literature revealed that there is no evidence of use of ammonium molybdate in abating salt stress. Ammonium is a source of nitrogen for protein synthesis and molybdenum is essential for nitrate reductase activity, chlorophyll synthesis and regulation of amino acid content. Young shoots and leaves of the plant studied are eaten. Young pods and seeds are used as vegetable and pulse. So the present work has been taken up to study the amelioration of sodium chloride stress by ammonium molybdate in *Vigna unguiculata*.

MATERIALS AND METHODS

The seeds of *Vigna unguiculata* (L.) Walp were used for the study. Different concentrations of sodium chloride (25 mM, 50 mM, 75 mM, 100 mM, 125 mM), ammonium molybdate (10 ppm, 15 ppm, 20 ppm 25 ppm, 30 ppm) and mixture of different concentrations of sodium chloride with 10 ppm concentration of ammonium molybdate in equal proportion (25mM + 10 ppm, 50 mM + 10 ppm, 75 mM + 10 ppm, 100 mM + 10 ppm, 125mM + 10 ppm) were prepared. Since higher concentrations of ammonium molybdate causes adverse effects only 10 ppm was used for further studies.

Seeds of uniform size were selected, treated with 0.1 percent mercuric chloride for one minute, washed thoroughly with water, placed equidistantly in sterilized petri dishes lined with filter paper and subjected to different treatments. In the first set of experiments, the seeds were moistened daily with 10 mL each of different concentrations of sodium chloride. In the second set, the seeds were moistened with 10 mL each of different concentrations of ammonium molybdate. In the third set the seeds were treated with 10 mL each of the mixture of sodium chloride and ammonium molybdate. A control set moistened with equal amount of distilled water was also maintained for reference. Germination was observed after 48 hours and the germination percentage was calculated.

Pot experiments were carried out to study the growth parameters. The pots, each with 20 seeds, were treated daily with 100 mL each of distilled water, different concentrations of sodium chloride solution (50 mM, 75 mM and 100 mM), 10 ppm of ammonium molybdate and mixture of different concentrations of sodium chloride and 10 ppm ammonium molybdate (50 mM + 10 ppm, 75 mM + 10 ppm and 100 mM + 10 ppm). After 15 days the seedlings were removed from the pots, washed thoroughly and used for estimation. The length and dry biomass of seedlings were measured. The total chlorophyll content (Harborne 1984), starch (McCready et al. 1950) and total free amino acids (Moore & Stein 1948) of leaves of seedlings were estimated. The mean values of the three replicates were presented.

RESULTS AND DISCUSSION

Seed germination was 100 percent in control, 10 ppm, 25 mM, 25 mM + 10 ppm and 50 mM + 10 ppm treatments. In all other treatments, it decreased gradually with an increase in concentration of solutions. An increase in germination percentage of seeds treated with combination of sodium chloride and ammonium molybdate over the treatments of different concentrations of only sodium chloride was recorded (Table 1). The length, biomass, chlorophyll, starch and total free amino acid contents were maximum in the seedlings treated with 10 ppm ammonium molybdate followed by control. They were more in the seedlings treated with 50 mM + 10 ppm solution than those treated with all other solutions. The values of 50 mM + 10 ppm treatment were close to those treated with water. The values obtained were more in the seedlings treated with combined solutions (50 mM + 10 ppm, 75

Table 1 : Amelioration of sodium chloride stress in *Vigna unguiculata.*

First set		Second set		Third set	
Treatment Sodium chloride (mM)	Germination %	Treatment Ammonium molybdate (ppm)	Germination %	Treatment Sodium chloride + Ammonium molybdate	Germination %
Control	100	-	-	-	-
25 mM	100	10 ppm	100	25 mM + 10 ppm	100
50 mM	90	15 ppm	94	50 mM + 10 ppm	100
75 mM	82	20 ppm	90	75 mM + 10 ppm	90
100 mM	76	25 ppm	86	100 mM + 10 ppm	82
125 mM	70	30 ppm	74	125 mM + 10 ppm	78

Note : mM = millimole; ppm = parts per million.

Table 2 : Amelioration of sodium chloride stress in *Vigna unguiculata.*

Treatment	Seedling length	Seedling dry biomass cm / Plant	*Total (g/Plant)	*Starch chlorophyll	*Total free amino acids
Control	24.124 ± 1.654	0.325 ± 0.015	1.576 ± 0.068	5.620 ± 0.085	2.807 ± 0.044
10 ppm	25.435 ± 1.903	0.358 ± 0.012	1.672 ± 0.075	6.351 ± 0.141	3.087 ± 0.095
50 mM	21.323 ± 2.006	0.302 ± 0.016	1.368 ± 0.056	4.984 ± 0.090	2.386 ± 0.052
75 mM	17.712 ± 1.982	0.242 ± 0.021	1.196 ± 0.036	3.633 ± 0.112	2.195 ± 0.084
100 mM	16.909 ± 1.096	0.209 ± 0.025	0.936 ± 0.027	3.442 ± 0.095	2.046 ± 0.062
50 mM + 10 ppm	24.212 ± 1.982	0.344 ± 0.013	1.503 ± 0.081	5.406 ± 0.115	2.883 ± 0.096
75 mM + 10 ppm	20.910 ± 1.982	0.306 ± 0.018	1.181 ± 0.064	4.861 ± 0.186	2.543 ± 0.084
100 mM + 10 ppm	18.415 ± 1.080	0.281 ± 0.011	1.086 ± 0.045	4.163 ± 0.101	2.240 ± 0.076

Note : Values are mean ± standard error; *mg/g fresh weight;
mM = millimole sodium chloride; ppm = parts per million ammonium molybdate

mM + 10 ppm and 100 mM + 10 ppm) than those treated with the respective pure sodium chloride solutions (50 mM, 75 mM and 100 mM) (Table 2).

The enhanced germination percentage of seeds treated with 50 mM + 10 ppm solution over 50 mM treatment may be due to reduction in the osmotic pressure of the solution by addition of ammonium molybdate. The inhibitory effect on seed germination by 50 mM and higher concentrations of sodium chloride must be due to the osmotic imbalance. Hampson & Simpson (1990) and Subburamu & Ramamoorthy (2003) have also recorded such findings.

The increased length, biomass, chlorophyll, starch and amino acid contents of the seedlings treated with 50 mM + 10 ppm must be due to stimulatory and antagonistic effects of ammonium molybdate. Sanwal & Varshney (1997) have attributed the improvement in growth characters by addition of ameliorant to the corrected soil osmotic balance due to soil nutrient level ultimately leading to stimulation in metabolic status of plants to variable extent. According to Deepak Bhat & Hosmani (1991) the reduction in length and biomass of ragi seedlings under sodium chloride stress must be due to decreased levels of endogenous growth regulators. Mashad & Kamel (2001) have also reported similar results in *Pisum sativum.*

The increase in chlorophyll, starch and total free amino acids contents of the seedlings treated with 50 mM + 10 ppm solution may be due to the stimulatory effect of molybdenum on enzyme activities, chlorophyll synthesis and nitrogen metabolism. According to Dwivedi et al. (1997) molybdenum plays an important role in chlorophyll biosynthesis. Cengiz Kaya et al. (2003) have reported similar results in salt stressed strawberry by addition of calcium nitrate and potassium nitrate. Reduction in the parameters studied at higher concentrations must be due to the synergistic effect of sodium chloride and ammonium molybdate. Similar findings were reported by Muthukumarasamy & Panneerselvam (1997) in *Pisum sativum* due to salt stress.

The present work suggests the use of low concentrations of ammonium molybdate in ameliorating salt stress in *Vigna unguiculata.*

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