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GROWTH RESPONSE OF *VIGNA AMBACENSIS* L. SEEDLING TO THE INTERACTION BETWEEN NITROGEN SOURCE AND SALT STRESS

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Abstract

Vigna ambacensis plants grown hydroponically in a Hoagland nutrient solution with either nitrate or ammonia as a source of nitrogen and NaCl a 0,40, 80 and 160 mM concentrations showed reduction in all growth parameters where ammonia was used as compared to nitrate as a nitrogen source. Salt decreased the growth of plants. Plants grown in nitrate were more salt tolerant as compared to ammonia especially at 80 and 160 mM NaCl level. High salinity conditions reduced calcium and potassium contents in shots with a increase in sodium which was higher (80 m μl) in NH^+_4-N as compared with NO^{-1}_3-N source.

Introduction

Salinization of soils is a serious problem in many areas of the world especially in semi-arid and arid regions where excessive soluble salts decrease the osmotic and water potentials of the root media and therefore impairs water uptake by roots. Reduction in growth of glycophytes under salt stress in terms of fresh weight and dry weight production have been reported (Hue & McCall, 1989. Pessarak *et al.*, 1989). Salt stress is reported to adversely affect plant metabolism and cause nutrient imbalance in Maccedamia seedlings (Hue & McCall, 1989) and *Phaseolus vulgaris* Seemann & Critchley, 1985). Nitrogen is also reported to affect salt stress tolerance in plants like chickpea (Lauter *et al.*, 1981), corn (Pessarak *et al.*, 1989) or wheat (Lewis *et al.*, 1989).

Nitrogen is a macro nutrient element required in plants through all growth stages of Nitrate and ammonium are the two major forms of nitrogen available for plant uptake. Most plants can absorb and utilize both forms of nitrogen, however a number of plant species have shown that growth on nitrate is more rapid than growth on ammonium as the sole nitrogen source (Smith *et al.*, 1990; Gentry *et al.*, 1989; Alfoldi & Pinter, 1992; Addition of small amounts of NH^+_4 to NO^-_3 has shown a increase in growth of tomato (Errebhi & Wilcox, 1990) as compared to nitrate alone Herber & Below, 1989). Nitrogen has a significant influence on numerous physiological mechanisism and on the absorption of other ions (Lips *et al.*, 1990; Magalhaes & Wilcox, 1983) Ammonium nitrogen nutrition is associated with reduced cation uptake as compared to nitrate nutrition (Magalhaes & Wilcox, 1983; Mackalon & Sim, 1980).

Vigna ambacensis, an annual herb which grows in the south western region of Saudi Arabia, is cultivated for seeds as a source of food. The present study was conducted to evaluate the response of this species to the interaction of salt stress and nitrogen source.

Materials and Methods

Seeds of *Vigna ambacensis* were collected from plants in the Gizan district, south western of Saudi Arabia and stored at 10°C until used. Prior to germination studies the seeds were allowed to imbibe water overnight at 25°C and then placed on the surface of two layers of Whatman No.1 filter paper moistured with distilled water petridishes. The dishes were placed in an incubator at 26°C under darkness. After 3 days of germination, four uniform seedlings were transferred to 2-L polyethylene container wrapped with aluminum. The containers were filled with half strength Hoagland nutrient solution containing either Ca $9NO_3)_2$ or KNO_3 or $NH_4)_2$ SO_4 as a source of nitrogen pH was adjusted to 6 by adding drops of diluted NaOH solution. The solution was continuously aerated. The containers were placed in a growth chamber set at 30/25°C day/night temperature and 14/10h day/night cycle with light intensity of 900 lux. The level of the nutrient solution in each container was maintained at 2 L throughout the experimental period by adding distilled water. Nutrient solution was replaced with a full strength Hoagland nutrient solution one week after transplanting with 40 mM NaCl added to each container except in the control. Concentration of NaCl was increased 40 mM daily till the desired concentration (up to 160 mM) was attained. Four replicates were used per treatment and the nutrient solution was changed weekly.

Seven weeks after completion of salt treatment, the plants were harvested and separated in to roots stems and leaves. The length of roots and stems were recorded and the total leaf area of each plant was measured. Fresh weight of each plant part was recould and dry weight was obtained after oven drying at 85°C to a constant weight. Dry samples of leaves, and stems were ground into a fine powder separately and digested using sulphuric acid and a mixture of hydrogen peroxide and perchloric acid 1:1 v/v and assayed for minerals ions using atomic absorption spectrophotometer (Model Pye Unicam-Sp. 6560 UV/VIS). Phosphorus was determined calorimetrically suing Ascorbic acid method (Watanabe & Olsem, 1965). Total nitrogen was also determined by the microkjeldahl technique (Jackson, 1958).

Results

Low NaCl concentration (40 mM) had no effect on plant survival in NO^-_3 - grown plants which reduced to 63% in NH^+_4 grown plants (Fig.1). At 160 mM, 50% of NO^-_3 grown plants survived till the end of the experimental period, while all the NH^+_4-grown plants died few days after the completion of salt treatment.

Nitrogen form had a significant effect on fresh weight and dry weight production of all plants parts organs (root, stem and leaves) (Table 1). Plant growth in terms of fresh weight and dry weight was lower in NH^+_4 grown plants as compared to NO^-_3 grown plants. Total fresh and dry weight of NH^+_4-grown plants was 69.7% and 76.8% of that of NO^-_3 grown plants, respectively. In both NO^-_3 - and NH^+_4 - grown

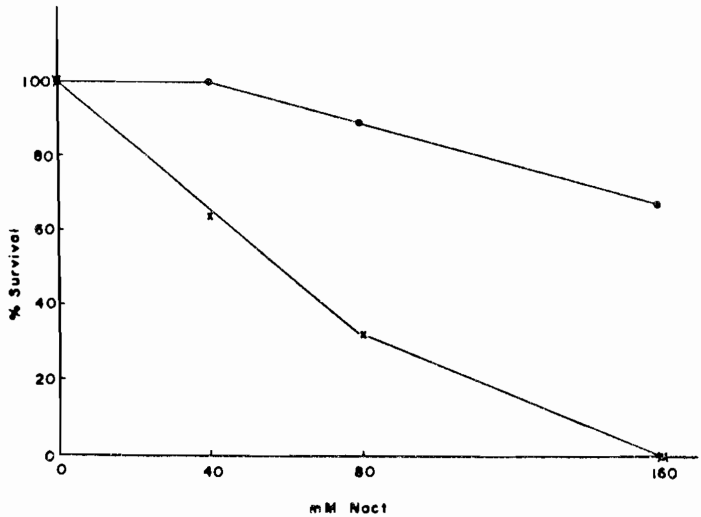

Fig.1. Effect of interaction between salt stress and nitrogen source on plant survival.

o ------------------- o nitrate

x ------------------- x ammonium

plants, the addition of NaCl reduced total plant fresh weight where salinity of 40 mM slightly increased the fresh weight of NH^+_4-grown plants. At 40 mM NaCl, total fresh weight of NO^-_3 - grown plants was lightly lower (-5%) than that of NH^+_4 - grown plants whereas at 80 mM NaCl, total fresh weight of NH^+_4 - grown plants was only 77% of that of NO^-_3 - grown plants. In NO^-_3 grown plants, total dry weight declined to 56.8, 49.6 and 25.2% at 40,80 and 160 mM salt concentration, respectively, whereas, in NH^+_4 - grown plants, total dry weight reduced to 80.4 and 46.3% at 40 and 30 mM NaCl, respectively.

Fresh and dry weights of root were not affected by salt concentration up to 80 mM and in some cases the weights slightly increased as compared to control plants. At 160 mM NaCl, root fresh and dry weight reduced significantly in NO^-_3 - grown plants to 50.9 and 45.6% respectively as compared to control plants. The shoot: root ratio in both ammonium and nitrate fed-plant, decreased respectively in response to salinity, indicating that shoot growth was more severely affected by salinity than root growth. Nitrogen form growth media also influenced the length of root and stem and total leaf area Root/Stem length and total leaf area were lower in NH^+_4 - grown plants as compared to NO^-_3 grown plants. Root length, stem length and total leaf area were respectively reduced by 76.7%, 68.9 and 78.8% in NH^+_4 - grown plants (Table 2).

Salinity also had some effects on the mineral compositions as influenced by nitrogen source. A decrease in shoot mineral contents, especially for calcium potassium phosphorus and nitrogen of both nitrogen treatment as a result of the increase in salinity level, was observed (Table 3) Sodium shoot content increased from 144 ppm at 0.0 NaCl level to 606 at 80 mM in nH^+_4-N treatment, and from 81.4 ppm at 0.0

Table 1. Effect of nitrogen source and NaCl concentration on fresh and dry weight of *Vigna ambacensis*.

Nitrogen Source	NaCl Conc. (mM)	root x±S.D	stem x±S.D	leaves x±S.D	shoot	Total	shoot root ratio
			Fresh weight (g/plant)				
Nitrate	0.0	0.55 0.014	1.81 0.02	1.73 0.05	3.54	4.09	6.4
	40.0	0.57 0.01	1.20 0.01	0.96 0.04	2.16	2.73	3.8
	80.0	0.53 0.01	0.90 0.01	0.81 0.04	1.17	2.24	3.2
	160.0	0.28 0.005	0.41 0.05	0.04 0.02	0.81	1.09	2.8
Ammonia	0.0	0.38 0.002	0.14 0.01	1.17 0.03	2.31	2.85	6.1
	40.0	0.46 0.002	0.14 0.01	1.30 0.03	2.44	2.90	5.3
	80.0	0.39 0.002	0.50 0.05	0.78 0.02	1.34	1.73	3.4
	160.0						
			Dry weight (mg/plant)				
Nitrate	0.0	29.4 1.0	182.1 2.0	16.0 5	242.1	371	8.23
	40.0	29.3 1.0	93.3 1.0	88.2 3	181.5	210.8	6.19
	80.0	29,3 1.0	74.9 1.0	79.8 3	153.7	184	5.25
	160.0	13.4 0.6	81.0 0.5	49.0 2	80.0	93.4	5.9
Ammonia	0.0	22.5	12.9 1.0	134 3	26.3	285	11.9
	40.0	28.5 0.8	79.3 0.5	121 3	200.3	229	7.03
	80.0	21.2 0.8	38.8 0.05	72 2	110.8	132	5.23
	160.0						
			Fresh weight (%)		Dry weight (%)		
Nitrate	0.0		---		---		
	40.0		66.75		66.82		
	80.0		54.77		19.60		
	160.0		26.65		25.18		
Ammonia	0.0		-		-		
	40.0		101.75		80.35		
	80.0		60.70		46.32		
	160.0						

in NaCl level to 487 pp at 80 mM NO_3-N indicating more (25% high) accumulation of sodium cation in NH^+_4-N treated plants. A slight difference in potassium and phosphorus ions was observed with both nitrogen sources (Table 3). However, shoot calcium content at NO^-_3-N source was twice more than that of NH^+_4-N source.

Table 2. Effect of nitrogen source on root and stem length and total leaf area.

Nitrogen source	NaCl Conc. (mM)	Root Length (cm) x±S.D.	Stem Length (cm)x±S.D.	Total Leaf Area (cm) x±S.D.
Nitrate	0.0	22.80 1.5	62.92 2	132.27 3
	40.0	18.25 0.7	31.5 2	69.02 2
	80.0	20.79 0.7	18.33 0.5	52.89 1.5
	160.0	17.5 0.6	11.81 0.4	---
Ammonia	0.0	17.54 0.5	18.17 3	104.19 4
	40.0	19.9 0.8	18.43 0.6	85.8 2.5
	80.0	19.3 0.6	12.2 0.5	10.97 1.0
	160.0			

Discussion

The present study showed that both the nitrogen forms (nitrate and ammonium) caused differences in all growth parameters of *Vigna ambacensis* Growth greatly was more reduced in NH^+_4 - grown plants as compared to NO^-_3 - grown plants which is similar to in pearl millet (Smith *et al.*, 1990) wheat Gentry *et al.*, 1989); tomato (Magalhaes & Wilcox, 1983) and maize (Alfoldi & Pinter, 1992). Reduction of plant growth by NH^+_4 as compared to NO^-_3 could be due to an inadequate accumulation of mineral cations and organic anions (Salsac *et al.*, 1987) or might due to a reduction in water uptake which may involve the effect of NH^+_4 on membrane permeability

Table 3. The effect of salinity levels and nitrogen sources on mineral composition in shoot of *Vigna ambacensis*.

Nitrogen Source	Salt level (mM)	Element concentration (ppm) Ca	K	Na	P	N(%)
$(NH_4)SO_4$	0.0	131.0	612.0	144.0	676.0	24.2
	40.0	126.7	712.0	416.0	656.0	28.1
	80.0	87.6	520.0	606.0	608.0	27.5
	160.0					
KNO_3	0.0	313.0	761.0	81.4	680.0	16.1
	40.0	311.8	639.0	267.7	644.0	17.3
	80.0	270.0	586.7	487.8	548.0	15.6
	160.0	163.3	602.9	517.0	481.0	16.2

(Macklon & Sim, 1980). Water stress has also been observed in plants supplied with NH^{+}_{4} (Quebedaux & Ozburn, 1973;

Addition of NaCl to the nutrient solution decreased the growth of *V. ambaceusis* either grown in NO^{-}_{3} or NH^{+}_{4} as a source of nitrogen. Reduction of plant growth under salt stress is usually attributed to osmotic stress due to external water potential, specific ion effect on metaboic process, or due to nutrient deficiency Seemann & Critchley, 1985; Hue & McCall, 1989). Reduction in growth of *Vigna ambacensis* by salt stress may not be due to Na^{+} toxicity only since growth was more adversely affected in NH^{+}_{4} - grown plants than NO^{-}_{3} - grown plants and NH^{+}_{4} - grown plants should accumulate more Na^{+} than NO^{-}_{3} - grown plants to help in regulating the cytoplasmic pH because the No^{-}_{3} - grown plants grow faster than NH^{+}_{4} - grown plants. than NO^{-}_{3} grown plants and NH^{+}_{4} - grown plants should accumulate more Na^{+} than NO^{-}_{3} - grown plants to help in regulating the cytoplasmic pH because the NO^{-}_{3} - grown plants grow faster than NH^{+}_{4} - grown plants. NH^{+}_{4} - grown plants were more sensitive to NaCl salinity than NO^{-}_{3} - grown plants especially at 80 and 160 mM, because NH^{+}_{4} ions compete with the absorption of other cations. This preassembly leads to a reduction in the leaf area of the plants and consequently in sufficient carbohydrates production to support growth. Similar results have been reported for rice (Youngdahl *et al.*, 1982). Where the toxic effect of the NH^{+}_{4}-N on plant growth attributed to factors such as interference of NaCl ions with NH^{+}_{4} assimilation in the root, deficiencies in the plant and acidification of the root environment. The result of the present study is in agreement with those of Lewis *et al.*, (1989) and Leidi *et al.*, (1992) that NH^{+}_{4} - N grown plants were highly susceptible to salinity. Higher growth rate of NO^{-}_{3} - plants might increase salt stress tolerance due to the dilution and distribution of absorbed toxic ions to various plant parts by faster growth. Better growth of NH^{+}_{4} grown plants at 4-0 mM NaCl as compared to NO^{-}_{3}-plants at the same salt level might he part due the less competition between plants for nutrients since 30% of the plants died whereas all NO^{-}_{3} - grown plants survived till the end of the experimental period.

Under high salinity conditions, plants generally have reduced root is Okusnya, 1979, 1980). Poor root growth may result from the reduction in the length of the root tip elongation zone, the length of the epidermal cells and a decline in the rate of cell production (Zidan *et al.*, 1990). The reduction in root growth lead to a decrease in its surface area, and therefore, nutrient uptake, higher water potential which restrict better root and shoot growth and thus *Vigna* seedling may not be able to adjust osmotically at high salinity level which is similar to the observations of Greenway (1968() for glycophytes.

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