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THE INFLUENCE OF NITRATE SUPPLY ON GROWTH AND *IN-VIVO* NITRATE REDUCTASE ACTIVITY OF *LEPTOCHLOA FUSCA* SEEDLINGS

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Abstract

Leptochloa fusca (L.) Kunth., seedlings supplied with 0, 5, 10 and 20 mM of nitrate were grown hydroponically as well as in sand culture. Fresh and dry weight of seedlings sampled at 10, 12, 14 and 16 days after plantation were significantly different from the zero nitrogen treatment with maximum increase in seedlings grown in 10 mM, NO_3 solution. Total-N of plants exhibited similar pattern. Nitrate reductase activity was high and comparable activities were obtained in shoots as well as in roots. Differences in all parameters were also influenced by the growing conditions.

Introduction

Nitrate (NO_3) is the common form of inorganic-N available to plants under normal field conditions. Inside the plant, nitrate is converted into ammonia (NH_3) by the action of assimilatory nitrate reductase and nitrite reductase (Beevers & Hageman, 1969). Nitrate reductase (NR) is widely distributed enzyme and has been isolated from microorganisms and from higher plants (Beevers & Hageman, 1980; Guerrero *et al,* 1981). As nitrate reductase is relatively unstable and the first enzyme in the sequence of reactions which incorporates assimilated nitrogen into proteins, the level of NR should reflect the rate of supply of reduced nitrogen for plant growth (Brunetti & Hageman, 1976).

Leptochloa fusca (L.) Kunth., (Syn. *Diplachne fusca* (L.) Beauv.) commonly known as Kallar grass is recommended as primary colonizer for salt affected soils (Sandhu & Malik, 1975). These soils are low in organic matter contents (Malik, 1980) and turn over rate of this meager fraction in our arid soils is higher as compared to temperate soils. The nitrate utilizing ability of this grass at different nitrate levels is presented.

Materials and Methods

Leptochloa fusca seeds surface sterilised in 2% streptomycin sulphate solution having 0.5 ml (v/v) detergent for 2 hrs and then in 0.2% $HgCl_2$ for one hr. were washed with sterilised distilled water and placed in 1.5% water-agar plates. Petri plates were kept in growth chamber at 26°C ± 2 for 4 days with 30,000 Lux light intensity and 70% relative humidity. Germinated seedlings were transferred aseptically to culture tubes (33x3 cm) fitted with special autoclavable silicone stoppers (Shinetsu Polymer Inc.)

and to 15x12 cm 2 liter pots containing autoclaved sand. They were supplied with half strength Hoagland's solution pH 6.8 with 5, 10 and 20mM Nitrate (NO_3) concentrations. N-free controls were supplied with culture solution in which KNO_3 and Ca $(NO_3)_2$ were respectively replaced by KCl and $CaCl_2$. Fifty ml of 1/2 strength Hoagland's solution was placed in each culture tube containing 2 seedlings per tube while double amount of solution was provided to each pot containing ten seedlings. They were placed in a growth chamber having day temp. 28°C ± 2 (16 hrs), night temp. 24°C ± 2 (8 hrs), relative humidity 70% and light intensity of 30,000 Lux.

After transplantation of 10, 12, 14 and 16 days, fresh and dry weight of root and shoot (leaves + stem) were taken and their total nitrogen analysed by Kjeldahl method (Bremner, 1965).

In-vivo nitrate reductase activity in root and shoot was measured by a modified method of Brunetti & Hagemann (1976). Plant parts (100 mg) were cut into small pieces with a razor blade and vaccum infiltrated in 10 ml extractant solution containing 0.1 M potassium phosphate buffer (pH 7.5), 0.05 M KNO_3 and 1% 1-n-propanol. All procedures upto incubation were carried out in a cold room (4°C) and glassware was kept in ice. Serum stoppered flasks were then flushed with nitrogen gas and incubated in dark at

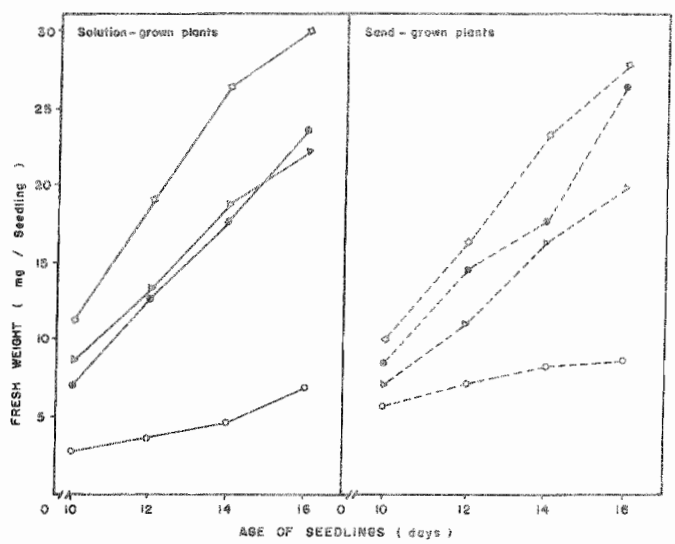

Fig. 1. Effect of nitrate on fresh weight of Kallar grass seedlings grown hydroponically (solid lines and in sand culture (broken lines) supplied with half strength Hoagland solution containing O mM to 20 mM nitrate (o —— o), O mM; (△ —— △), 5 mM; (□ —— □) 10 mM; (● —— ●) 20 mM NO'_3.

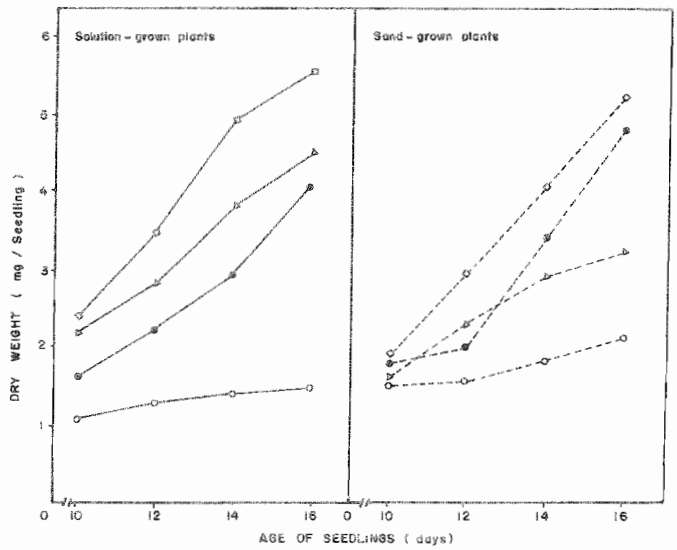

Fig. 2. Effect of nitrate on dry weight of Kallar grass seedlings. Conditions and symbols are similar as for Fig. 1.

30°C. Aliquots of one ml were taken at zero time and after 30 and 60 minutes. One ml of test sample was mixed with one ml of 1% (w/v) of sulphanilamide (Sigma) in 3M HCl and one ml of 0.1% N- (1-napthyl) ethylenediamine di HCl. Samples were incubated at 30°C for 30 minutes and colour intensity measured at 540 nm on a Beckman Model-25 spectrophotometer. Concentration of nitrite was calculated from the standard curve of sodium nitrite solution. Readings are expressed as μ moles of NO_2-N gram fresh weight^{-1} hour^{-1}

Results and Discussion

NO_3 supply significantly increased plant weight (Fig. 1 & 2). Maximum fresh and dry weight was observed in 10 mM NO_3 treatment and higher level of NO_3 (20 mM) showed an adverse effect on the growth of Kallar grass seedlings especially in solution grown plants. Supra optimal nitrogen (nitrate) supply is reported to upset the balance between nitrate and nitrite reductase and caused some nitrite accumulation in *Lolium* (Goodman *et al* 1974). Sattar & Ahmad (1979) have observed an appreciable increase in growth of jute seedlings but with less difference between zero and 10 mM NO_3-treatments. The difference is more pronounced in Kallar grass as their seeds are very small (260 μg/seed) and unable to supply stored reserve material for a longer period. Growing conditions had a marked effect on the growth of Kallar grass seedlings since solution grown plants gained more weight as compared to sand grown plants. Similar effect was observed by Dale (1976) on barley seedlings.

Table 1. Total-N contents of Kallar grass seedlings grown under various nitrate levels.

No.	NO_3 levels	Growth conditions	Total-N µg/seedling				% N			
			10	12	14	16	10	12	14	16
1a		Solution	12.72	12.09	14.58	14.72	1.2	0.9	1.08	1.03
	0 mM									
b		Sand	19.11	18.84	21.06	23.32	1.30	1.24	1.17	1.09
2_a		Solution	38.88	46.20	60.04	64.35	1.80	1.65	1.58	1.48
	5 mM									
b		Sand	27.10	33.28	46.52	48.0	1.76	1.46	1.61	1.50
3a		Solution	39.84	60.03	63.10	82.14	1.69	1.74	1.30	1.48
	10 mM									
b		Sand	33.83	49.56	64.16	72.40	1.79	1.68	1.60	1.39
4_a		Solution	26.79	40.66	49.09	60.55	1.74	1.90	1.67	1.51
	20 mM									
b		Sand	31.68	36.43	60.20	81.12	1.80	1.84	1.72	1.69

Total-N was 2-3 folds higher in nitrate treated plants as compared to control (Table 1). With increase in nitrate supply total-N also increased and the effect was more pronounced in solution grown plants except in 20 mM NO_3 treatment where total-N of solution grown plants was lower. No such effect was observed in plants grown in sand. Uptake of NO_3 is strongly influenced by its own concentration, pH and supply of oxygen (Huffaker & Rains, 1978). Moreover at high-N concentration root development is also retarded which results in decreased nutrient uptake. Such effect was more pronounced in solution culture as compared to sand cultures. Supra optimal level of nitrate and anaerobiosis may be responsible for low total-N in solution grown plants.

The determination of *in-vivo* activity of nitrate reductase (EC. 1.6.6.1) is dependent on buffer concentration, exogenous nitrate concentration, anaerobosis and pH of extracting medium (Jaworaki, 1971; Brunetti & Hageman, 1976 and Yoneyama, 1981). In order to optimize the extracting conditions for Kallar grass, effect of pH and NO_3 concentrations was observed before the experiment. (Figs.3a & b.) 50 mM NO_3 concentration in extracting buffer gave maximum value while pH 7.5 was found to be optimum.

Table 2. Effect of nitrate supply on nitrate reductase activity of *Leptochloa fusca* (Kallar grass) seedlings

No.	Treatment	Growing Media	Plant material	Nitrate reductase umoles/gm fr.wt.xhr			
				DAP			
				10	12	14	16
1.	0 mM	Solution	Shoot	0.57	0.41	0.51	0.58
			Root	0.10	0.08	0.06	0.14
		Sand	Shoot	0.51	0.74	0.74	0.80
			Root	0.40	0.56	0.58	0.59
2.	5 mM	Solution	Shoot	1.01	1.09	1.18	1.36
			Root	0.79	1.28	1.44	1.36
		Sand	Shoot	0.76	1.23	1.34	1.54
			Root	0.51	0.59	0.83	0.78
3.	10 mM	Solution	Shoot	1.22	1.15	1.43	1.01
			Root	0.88	1.23	1.41	1.07
		Sand	Shoot	1.09	1.25	1.94	1.87
			Root	0.83	0.65	0.67	0.81
4.	20 mM	Solution	Shoot	1.04	1.45	1.22	0.91
			Root	0.57	0.77	0.79	0.93
		Sand	Shoot	1.32	1.43	1.30	1.34
			Root	0.78	0.86	0.69	0.74

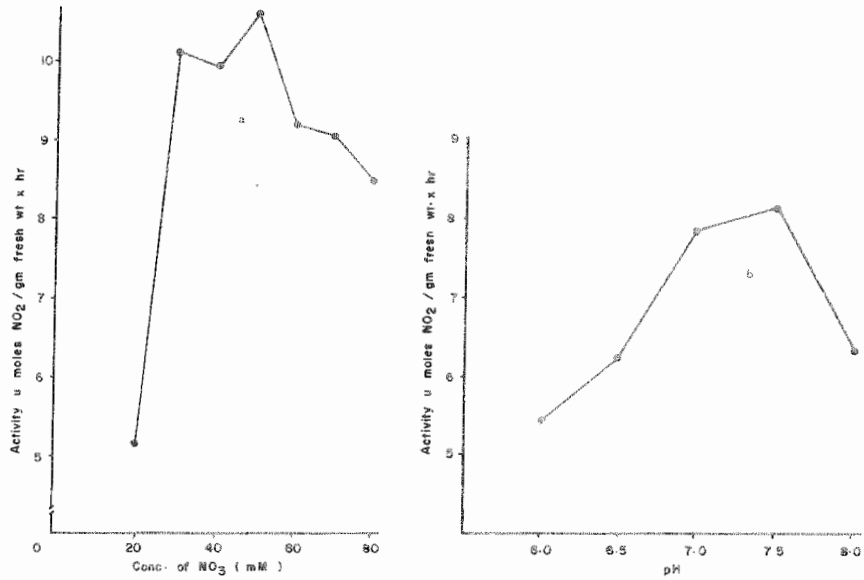

Fig. 3a. Effect of nitrate concentration on nitrate reductase activity in Kallar grass leaf. pH of phosphate buffer (0.1M) was 7.5.

b. Effect of pH on nitrate reductase activity in Kallar grass leaf. The concentration of NO_3 and phosphate buffer was 50 mM and 0.1 M respectively.

Supply of nitrate resulted in significant increase of NR-activity (Table 2). In a study on several grasses and cereals grown in sand culture, Goodman *et al* (1974) observed low NR activities when compared to the present values. Moreover, appreciable *in-vivo* activities were present in roots of Kallar grass seedlings. Redinbaugh & Campbell (1981) while studying the nitrate reductase activity in roots of maize emphasized the need to study the contribution of the root NR activity to the total nitrogen economy of plant. Sattar & Ahmad (1979) measured the *in-vivo* NR activities of different parts of jute seedlings and observed that activities were lower when measured after 7 days of growth. However, NR activity was higher in kallar grass seedling at different times of measurements indicating the depletion of organic nitrogen (7.07 μg-N/seed) which was unable to represes the nitrate reductase activity even at the time of first analysis.

Substantial amount of nitrate reductase activity is mostly located in the leaves of *Leptochloa fusca.* Studies on different physiological factors affecting nitrate reductase activity in germinating seedlings of Kallar grass are in progress.

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