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EFFECT OF COMBINED NITROGEN ON GROWTH AND NODULATION OF TWO MUNGBEAN (*VIGNA RADIATA* [L.] WILCZEK) CULTIVARS

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Abstract: Two mungbean (*Vigna radiata* [L.] Wilczek) cultivars, NM-51 and NM-92, were compared for plant growth and root nodulation during development at different nitrogen regimes (0, 2, 5 and 10 mM) in sand cultures. Dry weight of plants, after 5, 8 and 10 weeks of sowing, increased in both cultivars at all the nitrate levels. The maximum increase in dry weight, however, occurred at 5 mM nitrate for NM-51 and at 2 mM nitrate for NM-92. The two cultivars were comparable in reproductive growth since number of flowers per plant, number of pods per plant and fresh weight of pods per plant generally increased with increasing nitrogen levels. Nodule development in both the cultivars was stimulated in the presence of 2 mM nitrate but was increasingly depressed by the higher levels of nitrate. Nitrate levels of 5 and 10 mM decreased nodule number and nodule fresh weight. Cultivar NM-92 showed increased growth and nodulation at all nitrate levels compared to NM-51. Also, nodulation of NM-92 was more tolerant to nitrate compared with NM-51.

Keywords: Biological nitrogen fixation**,** combined nitrogen, growth, mungbean, nodulation, *Vigna radiate*.

INTRODUCTION

Biological nitrogen fixation is of great practical importance since the potential environmental hazards of nitrogenous fertilizers have raised ecological concern and also the fertilizers are becoming steadily less economic [Sprent and Sprent 1990]. Most of nitrogen-fixing prokaryotes are free-living microorganisms found in the soil. A few form symbiotic associations with higher plants in which the prokaryote directly provides the host plant with fixed nitrogen in exchange for the nutrients and carbohydrates. Such symbiosis occurs in nodules that form on the roots of the plants and contain the nitrogen-fixing bacteria. The most common type of symbiosis occurs between leguminous plants and soil bacteria called *Rhizobium*. Agriculturally important legumes are thus estimated to account for about 50 % of the total nitrogen fixed by biological systems [Okon and Hardy 1983].

Symbiotic nitrogen fixation is the result of a delicate balance between a higher plant and specific bacteria. It is important to understand properly the optimum conditions for this fixation in order to provide full benefits to the plant. Stress conditions of various kinds are known to affect symbiotic nitrogen fixation [Sprent and Sprent 1990, Stacy *et al*. 1992]. Several studies have shown that the process of nodulation may be promoted by relatively low concentrations of available nitrate or ammonium, but higher concentrations of which are almost always inhibitory [Herdina and

Salisbury 1989, Buttery *et al*. 1990, Wu and Harper 1990, Walsh 1995]. Other reports have suggested that the response of nodulation to combined nitrogen application is variable among legume species [Danso *et al*. 1990, Walsh 1995]. The present study was therefore carried out to investigate the effects of increasing concentrations of nitrate on growth and nodulation of two cultivars of *Vigna radiata*, NM-51 and NM-92.

MATERIALS AND METHODS

Seeds of mungbean (*Vigna radiata* [L.] Wilczek) cultivars NM-51 and NM-92 (obtained from NIAB, Faisalabad) were sown in plastic pots containing washed coarse sand and inoculated with *Rhizobium* strain (BioPower-Biofertilizer for legumes, NIBGE, Faisalabad). Four days after sowing, inoculation was repeated. The pots were watered to field capacity when required. A complete nitrogen-free nutrient solution [Meidner 1984] amended with different levels of nitrate was applied two times a week after flushing the pots with water to avoid accumulation of unabsorbed salts in the cultures. The nitrate treatments involved application of KNO_3 at 0, 2, 5 and 10 mM. The experiment was arranged in a completely randomized design. Six replicate plants of each cultivar were harvested for each nitrate treatment, after 5, 8 and 10 weeks of sowing when the mungbean plants were at the first flowering, beginning of pod growth and middle of pod growth stages respectively [Anonymous 1983].

RESULTS AND DISCUSSION

Growth of the two mungbean cultivars was compared during development at four levels (0-10 mM) of nitrate. Addition of nitrate increased shoot and root dry weights of both NM-51 and NM-92 at all three harvests (Figs. 1-3). The increase in dry weights was generally more prominent at 2 and 5 mM nitrate compared to that of 10 mM nitrate. The growth response of the two cultivars to nitrate, however, was different. At all nitrate treatments and each harvest, NM-92 generally showed higher dry weights compared to that of NM-51. Also, the highest increase in dry weights was observed at 5 mM nitrate for NM-51 and at 2 mM nitrate for NM-92 at all three harvests.

The two cultivars were comparable in reproductive growth since the number of flowers per plant, number of pods per plant and fresh weight of pods per plant generally increased with increasing levels of nitrate (Table 1).

Both the cultivars showed symptoms of nitrogen deficiency (indicated by brown pigmentation of leaves) at 0 mM nitrate throughout development. This suggested that the biological nitrogen fixation alone was insufficient to meet the nitrogen demand of the plants grown under the cultivation conditions of our experiment. Nitrogen deficiency symptoms were not observed in plants supplied with nitrate thereby confirming that the deficiency symptoms were due to nitrogen.

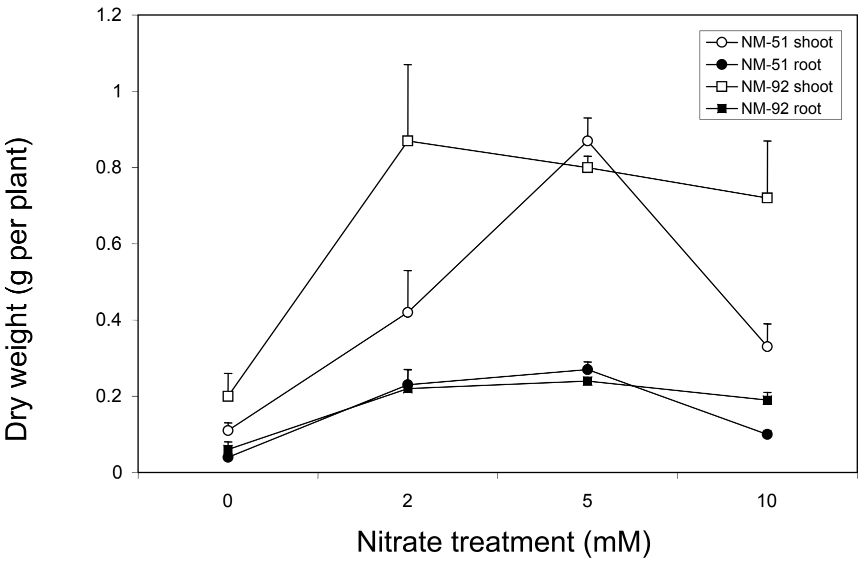

Fig. 1: Dry weight of shoot and root of mung bean cultivars NM-51 and NM-92 at different levels of nitrate after 5 weeks of sowing.

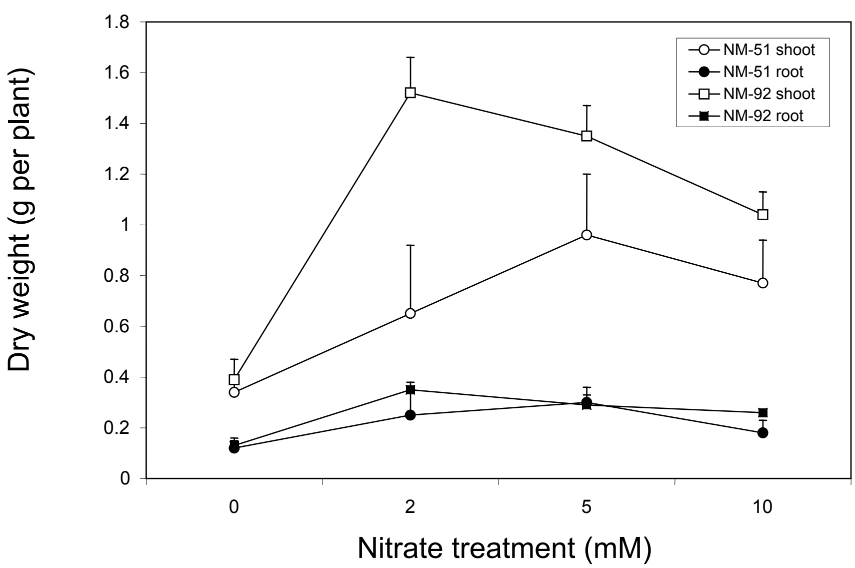

Fig. 2: Dry weight of shoot and root of mung bean cultivars NM-51 and NM-92 at different levels of nitrate after 8 weeks of sowing.

Root nodulation was characteristically promoted in both the cultivars in the presence of 2 mM nitrate but was increasingly depressed by 5 and 10 mM nitrate at each harvest (Table 2). The number of nodules per plant and fresh weight of nodules per plant increased at 2 mM nitrate but declined at 5 and 10 mM nitrate. At 10 mM nitrate, nodules were not formed with the exception of few nodules formed by NM 92 at the third harvest. At each nitrate concentration, the number of nodules per plant was consistently higher in NM-92 compared to that of NM-51. A similar trend was observed for the fresh weight of nodules per plant. This suggests that root nodulation of NM-92 was more tolerant to the nitrate levels compared to NM-51.

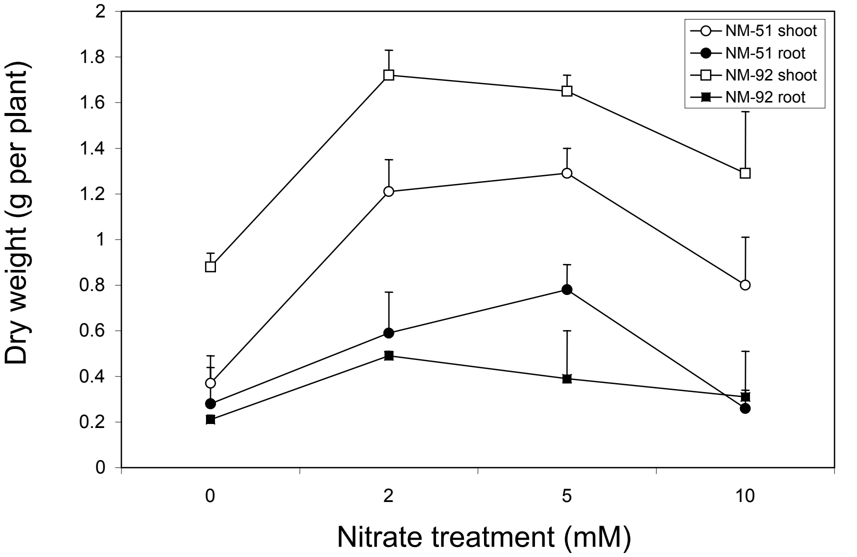

Fig. 3: Dry weight of shoot and root of mung bean cultivars NM-51 and NM-92 at different levels of nitrate after 10 weeks of sowing.

Table 1: Effect of nitrate levels on number of flowers, pods and fresh weight of pods of two mungbean cultivars (NM-51, NM-92).

Weeks after sowing	Nitrate treatment (mM)	NM-51 Number of flowers plant^{-1}	NM-51 Number of pods plant^{-1}	NM-51 Fresh weight of pods (g plant^{-1})	NM-92 Number of flowers plant^{-1}	NM-92 Number of pods plant^{-1}	NM-92 Fresh weight of pods (g plant^{-1})
5	0	0.17 ± 0.01	0.00	0.00	0.50 ± 0.12	0.00	0.00
	2	1.00 ± 0.30	0.17 ± 0.02	0.03 ± 0.01	1.33 ± 0.16	1.33 ± 0.24	0.14 ± 0.05
	5	1.17 ± 0.23	0.00	0.00	1.67 ± 0.21	0.83 ± 0.20	0.06 ± 0.02
	10	0.67 ± 0.09	0.00	0.00	1.50 ± 0.24	1.33 ± 0.26	0.13 ± 0.05
8	0	0.33 ± 0.07	1.16 ± 0.16	0.20 ± 0.06	0.44 ± 0.09	1.00 ± 0.13	0.17 ± 0.02
	2	1.20 ± 0.22	0.67 ± 0.24	0.27 ± 0.19	1.00 ± 0.21	1.67 ± 0.21	0.47 ± 0.10
	5	1.50 ± 0.48	1.50 ± 0.21	0.41 ± 0.03	2.67 ± 0.27	2.50 ± 0.27	0.74 ± 0.12
	10	0.68 ± 0.22	2.33 ± 0.22	0.58 ± 0.17	2.00 + 0.32	3.00 ± 0.13	1.00 ± 0.05
10	0	0.00	1.50 ± 0.11	0.44 ± 0.03	0.00	1.30 ± 0.16	0.59 ± 0.22
	2	0.66 ± 0.21	3.33 ± 0.47	0.89 ± 0.30	0.50 ± 0.17	2.50 ± 0.21	0.86 ± 0.24
	5	0.33 ± 0.08	5.00 ± 0.42	1.73 ± 0.27	0.56 ± 0.15	3.17 ± 0.23	1.06 ± 0.21
	10	0.00	0.83 ± 0.24	0.34 ± 0.12	0.66 ± 0.21	3.33 ± 0.27	1.05 ± 0.28

Each value represents mean of 6 replicates ± SEM.

Present results show that root nodulation of mungbean was remarkably increased at lower concentration of nitrate whereas it was strongly depressed at higher nitrate concentrations. The plant growth, however, was strongly promoted at all nitrate applications with maximum growth at the intermediate nitrate levels. These results can be compared with some other reports. Streeter [1988] documented nitrate inhibition of nodulation and nitrogen fixation in various leguminous plants. Naisbitt and Sprent [1993] reported that low concentration of nitrate stimulated nodulation in crop legumes. Herdina and Salisbury [1989] showed that nodule development in Faba Bean (*Vicia faba* L.) and Pea (*Pisum sativum* L.) was slightly stimulated in the presence of 2.5 mM nitrate, whereas both 5.0 and 7.5 mM nitrate delayed nodulation, decreased nodule number and decreased nodule activity but strongly promoted plant growth.

Table 2: Effect of nitrate levels on number and fresh weight of nodules of two mungbean cultivars (NM-51, NM-92).

Weeks after sowing	Nitrate treatment (mM)	NM-51		NM-92	
		Number of nodules $plant^{-1}$	Fresh weight of nodules (g $plant^{-1}$)	Number of nodules $plant^{-1}$	Fresh weight of nodules (g $plant^{-1}$)
5	0	1.33 $\pm$ 0.31	0.03 $\pm$ 0.01	3.67 $\pm$ 0.68	0.03 $\pm$ 0.01
	2	3.33 $\pm$ 0.67	0.04 $\pm$ 0.02	4.50 $\pm$ 0.54	0.05 $\pm$ 0.01
	5	0.00	0.00	0.17 $\pm$ 0.02	0.01 $\pm$ 0.01
	10	0.00	0.00	0.00	0.00
8	0	1.17 $\pm$ 0.36	0.05 $\pm$ 0.03	1.50 $\pm$ 0.32	0.05 $\pm$ 0.02
	2	2.78 $\pm$ 0.62	0.08 $\pm$ 0.03	2.84 $\pm$ 0.47	0.08 $\pm$ 0.03
	5	0.83 $\pm$ 0.27	0.01 $\pm$0.01	1.33 $\pm$ 0.40	0.03 $\pm$ 0.02
	10	0.00	0.00	0.00	0.00
10	0	0.66 $\pm$ 0.24	0.01 $\pm$ 0.01	2.00 $\pm$ 0.26	0.05 $\pm$ 0.02
	2	2.83 $\pm$ 0.60	0.06 $\pm$ 0.02	4.70 $\pm$ 0.37	0.09 $\pm$ 0.04
	5	0.50 $\pm$ 0.11	0.01 $\pm$ 0.01	1.78 $\pm$ 0.17	0.04 $\pm$ 0.02
	10	0.00	0.00	1.67 $\pm$ 0.55	0.03 $\pm$ 0.03

Each value represents mean of 6 replicates $\pm$ SEM.

The mungbean cultivar NM-92 showed increased growth and nodulation compared to NM-51 at all the nitrate levels. Also, nodulation of NM-92 was apparently more tolerant of nitrate compared to NM-51. The present results can be compared with other reports that suggested variable tolerance of nodulation to elevated levels of nitrate. Wu and Harper [1990] reported increased nitrate tolerance of several nitrate-tolerant soybean mutants. Hansen *et al*. [1989] reported that nitrate tolerant supernodulating (nts) mutants of soybean possessed higher nitrogen-fixing activity than the parent cultivar Bragg when exposed to elevated levels of nitrate. Wu and Harper [1990] confirmed earlier observations that three soybean nodulation mutants (NOD1-3, NOD2-4, and NOD3-7) were more tolerant to nitrate compared with the parent cultivar Williams.

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