Preliminary Studies on Nitrate Reductase and Nitrogenase Activities in Winged Bean (Psophocarpus Tetragonolobus (L)DC)

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Abstract

Two experiments carried out on the method of measuring nitrate reductase activity (NRA) in winged been indicated that the NRA values vary with the leaf fragment size, 2mm size recording the highest and anerobic conditions given during the incubation period of NRA assay did not affect the NRA value. In the third experiment carried out to investigate the variation in NRA, nitrogenase activity and nitrate assimilation capacity in winged bean during a selected date showed variation in all parameters, but significant differences were shown only in NRA measurements.

Introduction

The response of the winged bean plant to utilize nitrogen is determined by the ability of the plant to utilize both nitrate from the soil and atmospheric nitrogen. Nitrate, the primary source of nitrogen available from the soil, gets reduced by nitrate reductase enzyme in the plant. It has been estimated that winged bean can fix large amounts of nitrogen in the root nodule by nitrogenase enzyme, i.e., 750 kg N per Ha. per year. (Iruthayathas et al 1982). Studies on Soybean and Phaseolus bean plants have indicated that the two processes, nitrate reduction and nitrogen fixation, are successive events, each contributing at defined stages of plant growth (Harper et al 1972, Franco et al 1979). Hence preliminary experiments were carried out on the method of measuring nitrate reductase and the variation of nitrate reductase, nitrogenase activity and nitrate assimilation capacity of winged bean during the day time.

Materials and method

All experiments were carried out in a glass house at the laboratory of soil fertility and soil biology, K. U. Leuven, Belgium. The Rhizobium strain used to inoculate the plants was KUL-BH.

Experiment I

Previously germinated and inoculated winged bean plants were grown in plastic pots of 3.5 kg capacity filled with 3 kg sieved sandy soil. N, P, K was added at the rate of 10 kg N/ha, 55 kg P_2O_5/ha and 55 kg K_2O/ha. The pots were maintained at an optimum soil moisture regime, air relative humidity of 75%; temperature of 26°C/20° (day/night).

Uppermost fully expanded trifoliates at the pod filling stage were taken, superimposed and cut into 2mm, 3mm, 4mm and 5 mm pieces and the treatments were replicated 4 times. The NRA of leaf sections was determined by the invivo method described by Bar-Akiva et al (1970) and modified by Bradley (1980). Two leaf samples (100-200 mg) from each treatment were placed in tubes containing 4 ml of KNO_3 in buffer solution. Tubes were incubated in a water bath in dark at 30° C for an hour. Aliquots (0.5ml) were taken from each tube and the NO_3^- produced as determined by the intensity of the colour reaction.

Experiment II

Uppermost fully developed winged bean trifoliates were superimposed and cut into 2mm pieces to prepare 4 samples. Two were incubated with KNO_3 in buffer solution as in Experiment I. The other two tubes which contained KNO_3 in buffer solution and leaf samples were stoppered, air was evacuated and N_2 gas was introduced to create anaerobic conditions. Treatments were replicated 8 times and NO_2 produced in both treatments were determined.

Experiment III

Pregerminated inoculated winged bean seeds were planted in soil-filled 12 cm hard paper pots. N was added at the rate of 30 ppm after 2 weeks of seedling emergence. The experiment layout was a completely randomized block design replicated 4 times with provisions for 6 samplings to investigate the variation in NRA, nitrogenase activity and Nitrate assimilation capacity during the day. Samples were taken 4 weeks after seedling emergence on 21st March 1983.

The uppermost fully developed trifoliate along with the two trifoliates below were taken and nitrate reductase determinations were done as in experiment I for 1 set of samples while the other set of samples as incubated in a medium of phosphate buffer without KNO_3 (minus nitrate assay). Nitrate assimilation capacity was estimated by dividing the value of NRA by the value of NRA obtained without KNO_3 in the buffer (minus nitrate assay).

Nitrogen fixation was determined by the standard acetylene reduction assay.

Results and discussion

NRA of leaf fragment sizes varied significantly (Table 1). The smallest leaf fragment size (2mm) showed the highest value in NRA of all leaf fragment sizes. Bradley (1980) reported that the disc size of 2.5 mm gave highest values in NRA in winter wheat.

There was no significant difference in NRA measurements done in aerobic and anaerobic conditions though some scientists working on NRA assays reported high values with anaerobic conditions but with oxygen bubbling intermittently during the incubation period.

NRA, acetylene reduction and nitrate assimilation capacity varied with the time of the day (Table 2). Except for the little decrease in values at 11.30 a.m., activities of both enzymes concerned increased from 7.30 a.m. till 1.30 p.m. followed by a decrease thereafter.

NRA recorded highest value at 1.30 p.m. which was significantly different from other values obtained at other times. Harper et al (1972) studying the diurnal variation in NRA reported the highest values at around 1.00 p.m. in soybean. Nitrate assimilation capacity also showed variations with time. It could be attributed to the fact that the absorption of NO_3^- from the soil and nitrate reductase enzyme activity itself depend on the light intensity which varies during the day. High nitrate assimilation capacity values obtained during the experimental time indicates the limitation of nitrate reductase activity by the availability of NO_3^- in the leaves, the main site of NO_3^- reduction.

Maximum nitrogenase activity was observed at 1.30 p.m. though not significantly different from other values obtained during the day time. Nitrogenase and nitrate activity depends on photosynthetic production for their ATP and election donor requirements. Minchin et al (1974) indicated nitrogenase activity as being dependent on the supply of photosynthate. Hence it is possible that the midday period which normally records high light intensities might be causing increased photosynthate to support both nitrogenase and nitrate reductase activities.

TABLE 1. — Effect of leaf fragment size in nitrate reductase activity (NRA) pmol NO_2^- g fresh weight^{-1} hr^{-1}

	2mm	3mm	4mm	5mm	LSD
NRA*	7169	2869	1316	626	771

**Significant at 1% level.*

TABLE II —Variation in NRA (pmol NO_2g fresh weight^{-1}hr^{-1}) and C_2H_2 reduction (pmol plant^{-1}hr^{-1}) during the day time.

Time of the day	NRA*	C_2H_2 reduction	Nitrate assimilation capacity
7.30 a.m.	11325	1357	13.98
9.30 a.m.	13033	2592	14.18
11.30 a.m.	12918	2347	15.22
1.30 p.m.	18649	3425	9.55
3.30 p.m.	9987	2718	9.72
5.30 p.m.	7962	1781	9.54
LSD (P=0.05)	5505	NS	—

**Significant at 5% level.*

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