226 ± 43 μg/g reducing sugars. The sugars present in the root exudates were raffinose, lactose, glucose and fructose. The total aminoacid content was 18.6 ± 3.9 μg/g. Among the amino acids, serine/glycine, glutamine, alanine, phenyl alanine and isoleucine/leucine were detected. The exudate also contained 11.6 ± 3 μg/g phenol. Only succinic acid was found in the organic acid fraction of the root exudate. The nature and the amount of substances exuded by the roots of plants depend on the plant species[6,12], age and environmental condition under which it is grown. The root exudates may play a role in neutralizing the soil pH and altering the microclimate of the rhizosphere through the liberation of water and carbon dioxide. Normally, a coconut tree has 4000 to 7000 roots; each tree exudes large quantities of sugars and other compounds into the rhizosphere which may directly or indirectly influence the number and quality of the microorganisms in the rhizophere.

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NITRATE REDUCTION IN RELATION TO NITROGENASE ACTIVITY IN SUMMER MUNG (*VIGNA RADIATA*) AS INFLUENCED BY BACTERIAL INOCULATION AND PHOSPHORUS APPLICATION

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Biological nitrogen fixation and nitrate assimilation represent two major sources of reduced nitrogen for plant growth and seed yield in legume crops. Enzyme nitrogenase and nitrate reductase are responsible for the operation of these two systems in the legumes. In general, different legumes derive 25–80% of the total plant nitrogen from nitrogen fixation[1] and the additional source of nitrogen is thought to be the nitrate via the enzyme nitrate reductase[2]. Since nitrate is considered to be the primary source of nitrogen available from the soil, it was presumed that the characterization of both nitrogen fixation and nitrate reduction throughout the crop growth period would be highly useful for proper understanding of nitrogen contribution from the two important sources. Levels of nitrogen fixation and specific nodule activity were highly correlated with supply of phosphatic fertilizer[3] and bacterial inoculation[4]. There is, however, paucity of information on nitrate reduction in relation to nitrogenase activity in summer mung as influenced by bacterial inoculation and phosphorus application.

The experiment was conducted in a factorial randomized block design having four levels of bacterial seed inoculation (*Rhizobium*, *Rhizobium* along with *Azotobacter chroococcum*, *Rhizobium* along with *Azospirillum brasilense* and no inoculation) and three levels of phosphorus (0, 13 and 26 kg P/ha) with three replications. The soil of the experimental field was sandy-loam in texture and alkaline (pH 8.1) in reaction with low in organic carbon (0.41%) and available P (7.2 kg/ha) and medium in available K (159.3 kg/ha). The strain of *Rhizobium* was obtained from NifTAL Project, University of Hawaii, USA, while *A. chroococcum* and *A. brasilense* were obtained from the Division of Microbiology, IARI, New Delhi. Single superphosphate was used as the source of supplying phosphorus. Nitrogen and potassium were not applied. Nitrate reductase activity in the uppermost 2–3 fully expanded leaves was assayed at 25, 45 and 55 days after sowing following the *in vivo*

procedure[5] with modifications[6]. Nitrogenase activity was assayed using the C_2H_2 reduction method[7] in detached nodules at 25, 45 and 55 days after sowing. Nodules of three plants from each plot were weighed and incubated separately with acetylene gas at 30°C in 30 ml air-tight gas tubes for 1 hr. After incubation the amount of C_2H_4 produced was determined using a gas chromatograph (NUCON-5000) and the average values of three plants expressed both in terms of specific nitrogenase activity (ηM C_2H_4 mg^{-1} nodule fr. wt. hr^{-1}) and total nitrogenase activity (ηM C_2H_4 $plant^{-1}$ hr^{-1}) were statistically analysed. To study the relationship between nitrate reductase and nitrogenase activity at different crop growth stages simple correlation coefficients (r values) were worked out.

Bacterial inoculation significantly reduced the nitrate reductase activity at different stages over no inoculation (table 1). Maximum reduction was observed when the seeds were inoculated with both *Rhizobium* and *A. chroococcum*. These results clearly indicated that bacterial inoculation significantly reduced the utilization of soil NO_3–N as nitrate reductase activity depends on the utilization of NO_3–N[8]. The nitrate reductase activity of summer mung throughout the season showed the occurrence of two peaks of activity; one at early vegetative stage (25 days after sowing) and the second peak at pod-filling stage (55 days after sowing). These characteristics indicated that nitrate reductase plays an important role in the utilization or assimilation of NO_3–N from soil both before and after flowering. This was in close conformity with the findings of Franco *et al*[8].

Nitrogenase activity per plant due to bacterial inoculation increased significantly at 45 days over no bacterial inoculation. Maximum increase was observed when the seeds were inoculated with both *Rhizobium* and *A. chroococcum*. The increase in nitrogenase activity per plant was due to the increased nodule number and nodule dry weight (mg/plant) as the specific nitrogenase activity did not show any significant variation due to bacterial inoculation (tables 1 and 2). The increased nodulation in the presence of *A. chroococcum* might be due to production of auxins, which help in curing root hairs[9] and thereby increase the plasticity of cell wall, which facilitates the entry of bacteria into the root hairs[10]. The beneficial effect of *A. chroococcum* with *Rhizobium* may also be due to the prolonged survival of *Rhizobium* in the presence of large amounts of polysaccharide gums[11]. Increased nodulation due to seed inoculation with *Rhizobium* along with *A. chroococcum* or *A. brasilense* was also reported by several workers[10,12,13]. At late pod-filling stage (55 days) the number of nodules per plant, nodule dry weight (mg/plant) and nitrogenase activity per plant, however, did not show any significant variation due to bacterial inoculation. This may be due to the early leaf senescence and

Table 1 *Nitrate reductase and nitrogenase activity as influenced by bacterial inoculation and phosphorus application*

Treatments	Nitrate reductase activity ηM NO_2 formed g^{-1} fr.wt.hr^{-1}			Specific nitrogenase activity ηM C_2H_4 mg^{-1} nodule fr.wt.hr^{-1}			Nitrogenase activity ηM C_2H_4 $plant^{-1}$ hr^{-1}		
	25 days	45 days	55 days	25 days	45 days	55 days	25 days	45 days	55 days
Bacterial inoculation									
Rhizobium	347.0	153.9	247.0	0.091	0.148	0.078	45.8	159.7	62.4
Rhizobium + *A. chroococcum*	333.8	144.4	215.6	0.096	0.155	0.077	57.6	163.5	67.3
Rhizobium + *A. brasilense*	347.7	168.1	227.9	0.095	0.148	0.079	48.5	158.3	65.8
Control	407.9	241.4	286.7	0.092	0.138	0.078	42.4	135.1	61.2
S. E of mean (±)	11.2	12.7	10.4	0.002	0.004	0.003	3.0	6.2	3.1
C.D. at 5%	32.7	37.2	30.6	N.S	N.S.	N.S.	8.6	18.0	N.S.
Levels of phosphorus (kg P/ha)									
0	413.1	207.4	264.7	0.089	0.147	0.075	32.7	136.6	54.9
13	347.8	167.9	242.0	0.096	0.146	0.079	53.1	156.3	65.1
26	319.7	155.5	226.2	0.095	0.149	0.080	59.9	169.7	72.5
S. E of mean (±)	9.7	11.0	9.0	0.004	0.003	0.004	2.8	5.3	2.7
C.D. at 5%	28.4	32.2	26.5	N.S.	N.S.	N.S.	8.2	15.6	7.9

Table 2 *Nodulation of summer mung as influenced by various treatments*

Treatments	Number of nodules per plant			Nodule dry weight (mg/plant)		
	25 days	45 days	55 days	25 days	45 days	55 days
Bacterial inoculation						
Rhizobium	32.3	47.0	19.4	68.1	86.7	45.7
Rhizobium + *A. chroococcum*	36.5	52.1	21.8	72.8	89.8	47.1
Rhizobium + *A. brasilense*	32.6	47.6	27.9	73.8	83.5	48.3
Control	27.7	41.0	23.0	67.3	75.3	45.3
S. E of mean (±)	2.7	2.1	3.2	3.1	3.0	2.6
C.D. at 5%	7.9	6.2	N. S.	N. S.	8.7	N. S.
Levels of phosphorus (kg P/ha)						
0	22.8	30.2	19.2	45.6	60.4	36.5
13	33.5	49.7	24.6	75.7	89.6	49.1
26	40.5	60.9	25.3	90.2	101.5	54.2
S. E of mean (±)	2.3	1.8	2.8	2.7	2.6	2.2
C. D. at 5%	6.8	5.3	N. S.	8.0	7.5	6.6

nodule degeneration which occurred at that stage due to the very short duration nature of the crop. The seasonal distribution of nitrogenase activity revealed that the activity was low at early vegetative stage (25 days), reached a maximum at flowering (45 days) and declined thereafter at pod-filling stage (55 days). This showed that flowering stage is very important for nitrogen fixation in summer mung. Similar seasonal distribution of nitrogenase activity in *Phaseolus vulgaris* was noted by Franco *et al*[8].

Phosphorus application showed similar trend as that of bacterial inoculation in respect of both nitrogenase and nitrate reductase activity. It increased nitrogenase activity per plant and simultaneously also reduced the nitrate reductase activity irrespective of the stages studied (table 1). Application of 13 and 26 kg P/ha was not significantly different in respect of activity of these two enzymes but were significantly different when compared with no phosphorus application. The increase in nitrogenase activity per plant was due to increased nodulation (table 2). This was apparent from the specific nitrogenase activity which was not influenced by phosphorus fertilization. These results are in complete agreement with earlier findings[3]. The reduction in nitrate reductase activity may be ascribed to increased nitrogenase activity per plant as the reduction in nitrate reductase activity was always associated with the corresponding rise in nitrogenase activity per plant. Nodules being the sites for biological nitrogen fixation the nitrogenase activity per plant might have increased with the increase in nodule number and nodule dry weight.

The nitrate reductase activity at 25 days was highly positively correlated with its activity at 45 and 55 days. Similarly nitrogenase activity per plant at 25 days was also correlated with its activity at 45 days (table 3). These relationships indicated that irrespective of the different stages of crop growth, the bacterial inoculation treatments with different levels of phosphorus showed variation of a particular magnitude with regard to the activity of these two enzymes. The nitrate reductase activity was, however, negatively correlated with nitrogenase activity per plant which indicated an inverse relationship between the activity of these two enzymes (table 3). As biological nitrogen fixation increases the utilization of soil, NO_3–N decreases and vice versa. This might be the reason why supplemental nitrogen often fails to give the desired response in legumes.

The seasonal patterns of nitrate uptake and reduction as well as the patterns of nitrogen fixation in summer mung have thus suggested that the process of nitrate assimilation and nitrogen fixation are successive events, each contributing nitrogen at defined stages of plant development. Nitrate reduction appears to be more important both at preflowering and post-flowering stages, while the maximum nitrogen fixation takes place at flowering stage.

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Table 3 *Coefficient of correlation between nitrate reductase and nitrogenase activity at different crop growth stages*

Characters	Nitrate reductase activity 45 days	Nitrate reductase activity 55 days	Nitrogenase activity per plant 25 days	Nitrogenase activity per plant 45 days	Nitrogenase activity per plant 55 days
Nitrate reductase activity					
25 days	0.83**	0.80**	–0.69*	–0.61*	–0.52
45 days	–	0.89**	–0.54	–0.72*	–0.67*
55 days	–	–	–0.63*	–0.58*	–0.65*
Nitrogenase activity per plant					
25 days	–	–	–	0.72**	0.66*
45 days	–	–	–	–	0.70*

* Significant at 5% level; **Significant at 1% level.

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USE OF PHYTOHORMONES IN SYNTHESIS OF *BRASSICA NAPUS* L.

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ARTIFICIAL synthesis of *Brassica napus* from its diploid progenitors *B. campestris* and *B. oleracea* is receiving increasing attention in recent times[1–3]. However, because of interspecific incompatibility, the frequency of hybrids obtained has been low. Several *in vitro* methods such as ovary, ovule or embryo culture have been used to increase the frequency of hybrids[4,5]. But there are difficulties in following these methods caused by the low retention time of pollinated ovaries, small size and low number of fertilized ovules and specific osmotic and nutritional requirements of very small embryos. If ovaries, ovules or embryos can be retained on the plant and allowed to develop to a stage when *in vitro* methods can be more accessible, interspecific hybrids can be synthesized at a higher frequency. Phytohormones have been successfully used to this end in some interspecific crosses in *Arachis*[6]. In this paper the usefulness of phytohormones in the synthesis of *B. napus* from the cross *B. c.* ssp. *chinensis* × *B. o.* var. *capitata* is examined.

NAA, GA_3 and Kn were used singly and in combination (GA_3 + NAA and Kn + NAA) at concentrations of 10^{-5}, 10^{-5} and 10^{-6} M respectively. For single hormone treatments, a swab of cotton wetted with the hormone solution was wrapped around the ovary after pollination. A total of five applications of the hormone were given on alternate days. For the combination treatments, the pistils were treated alternately with each hormone over a period of 10 days. As control, the incompatibly