RESPONSE OF CHICKPEA (*CICER ARIETINUM* L.) TO INOCULATION AND NITROGEN FERTILIZER APPLICATION

L.G. Hernandez and G.D. Hill
Department of Plant Science, Lincoln College, Canterbury

ABSTRACT

The response of chickpea (*Cicer arietinum* L.) to added nitrogen fertilizer with and without inoculation was studied in a pot experiment. Plants inoculated with *Rhizobium* strain CC1192 were well nodulated. Applications of nitrogen decreased the number of nodules formed during the early stage of growth. Inoculation alone and in combination with nitrogen fertilizer increased dry matter production. Highest dry matter yields were obtained from inoculated plants treated with the equivalent of 30 kg N/ha 30 days after sowing. This suggests that the chickpea-rhizobia symbiosis does not fix enough nitrogen for maximum yield. This work indicates the need to inoculate chickpeas grown in soils without appropriate rhizobial population and also possibly nitrogen application in fields with low soil N status.

Additional Key Words: nodulation, timing, herbage yield

INTRODUCTION

Chickpea (*Cicer arietinum* L.) has the potential to become a new pulse crop in Canterbury. Initial research (Hernandez and Hill, 1983) has shown that chickpeas can be successfully grown in the region where they could fill the need for an alternative rotational crop with cereals.

When inoculated with an effective strain of *Rhizobium,* yield increases of 17 to 131% have been obtained (Sundara Rao and Sen, 1969). Symbiosis with *Rhizobium* provides a cheap source of nitrogen. However, the atmospheric nitrogen fixed may not be sufficient to supply the full nitrogen need of the crop. On the other hand, fertilizer nitrogen applications may reduce nodulation and nitrogen fixation (Sprent, 1979).

The timing and amount of supplementation are also critical in assessing the effects of nitrogen fertilizer. Stages considered most likely to benefit from applied nitrogen are either during the early seedling establishment before fixation commences (Pate and Dart, 1961) or during seed filling when there is a peak demand for nitrogen for synthesis of seed protein (Pate, 1976). Although positive responses to starter nitrogen dressing of about 15-25 kg N/ha have been reported for chickpea by several workers (e.g. Chundawat *et al.,* 1976; Rathi and Singh, 1976), little information is available on the effect of nitrogen application at a later stage of plant growth.

This study was conducted to investigate the effects of added fertilizer nitrogen on inoculated and un-inoculated chickpeas. Nodulation and dry matter production as affected by time of nitrogen application are also reported.

MATERIALS AND METHODS

Pre-germinated 'kabuli' type chickpea seeds were sown in 15 cm diameter plastic pots containing equal mixtures of autoclaved sand and vermiculite. Four seeds were sown to each pot and were thinned later to two plants per pot to maintain a uniform crop stand. Each pot was isolated in an individual plastic container to prevent *Rhizobium* contamination. The trial was set up in the Plant Science glasshouse, Lincoln College, in a 2 x 4 factorial with randomised complete block design replicated 12 times.

Solid inoculant of *Rhizobium* strain CC1192 obtained from Coated Seeds, Port Mapua, Nelson, was applied with the seed to half of the total number of pots at a rate equivalent to 40 kg/ha. Four nitrogen treatments (0, 30 kg N/ha as urea applied at sowing, 15 kg N/ha applied at sowing plus 15 kg N/ha applied 30 days after sowing, and 30 kg N/ha applied 30 days after sowing) were surface applied to both the inoculated and un-inoculated pots.

Each pot was irrigated every other day with 200 ml modified zero-N nutrient solution (Hoglund, 1973) to provide the necessary macro and micro-elements required for legume growth. Daily maximum and minimum temperatures in the glasshouse ranged from 25-30 °C and 15-18 °C, respectively. The photoperiod averaged 15.9 hours per day during the experiment.

Three replicates were harvested at 30, 45, 60 and 75 days after sowing and dry matter yield of roots and tops measured. Nodules were scored on a 0-5 scale following the classification of Corbin *et al.* (1977).

RESULTS

Slight chlorosis was observed in the zero-N and inoculation treatments during the early growth stages. Three weeks after sowing, inoculated plants had gradually changed to a normal green. The control plants however, remained chlorotic. At the later stages of growth when inoculation was effective, marked visual differences among treatments could be observed. Inoculated plants with or without nitrogen were greener than those without inoculation.

Nodulation

Thirty days after sowing, the inoculated plants were effectively nodulated (as indicated by the presence of red leghaemoglobin) while the un-inoculated plants showed no nodulation. Nitrogen applied at sowing significantly reduced the number of effective nodules formed (Table 1).

TABLE 1: Effect of nitrogen fertilizer on nodulation of inoculated chickpea, 30 and 45 days after sowing (DAS).

Nitrogen treatment*	Number of nodules formed	
	30 DAS	45 DAS
0 kg/ha	38.0 a**	49.6 a
30 kg/ha at sowing	23.5 b	50.5 a
15 kg/ha at sowing plus 15 kg/ha 30 days after sowing	28.0 b	59.2 a
30 kg/ha 30 days after sowing	35.5 a	52.0 a
S.E. (Mean)	1.65	0.02
C.V. (%)	11.0	15.7

* The un-inoculated treatments were not nodulated and not entered into the analysis of variance.
** Figures followed by the same letters in the same column are not significantly different at $P \leqslant 0.05$.

At flowering, 45 days after sowing, the un-inoculated plants still showed no nodulation. Among nodulated plants however, there was no significant difference in the number and dry weight of nodules formed. Similar observations on nodulation were obtained 60 and 75 days after sowing. Apparently the inhibition of nodulation by the nitrogen treatments occurred only at an early stage and the nodule mass of these plants was able to catch up in time with the control.

TABLE 2: Effect of inoculation and nitrogen fertilizer application on top dry matter yield of chickpea, 60 and 75 days after sowing (DAS).

Treatment	Top DM yield (g/plant)	
	60 DAS	75 DAS
Inoculation (I)		
Nil	1.92	1.92
Inoculated (40 kg/ha)	2.38	2.79
L.S.D. (5%)	0.404	0.376
Nitrogen (N)		
0 kg/ha	1.58 b	1.99 b
30 kg/ha at sowing	1.96 b	2.09 b
15 kg/ha at sowing plus 15 kg/ha 30 days after sowing	2.60 a	2.49 a
30 kg/ha 30 days after sowing	2.44 a	2.85 a
I x N	*	**
S.E. (Mean)	0.09	0.09
C.V. (%)	12.8	10.8

Dry matter production

Inoculation with *Rhizobium* improved dry matter yields but not until 60 days after sowing (Table 2). Inoculated plants had accumulated 19% and 31% more top dry matter than the un-inoculated plants at 60 and 75 days, respectively. Time of nitrogen fertilizer application also influenced top dry matter yields. At 60 and 75 days after sowing, plants treated with 15 and 30 kg N/ha at pre-flower (30 days) had significantly higher top dry matter yields than control plants and those which had received 30 kg N/ha at sowing. There was a highly significant ($P \leqslant 0.01$) inoculation by nitrogen interaction in top dry matter yield (Table 3). Without inoculation, top dry matter from nitrogen-treated plants was higher than that from the control, with no differences among the nitrogen treatments. With inoculation, plants given 30 kg N/ha 30 days after sowing had a top dry matter yield of 3.5 g/plant which was significantly higher than the 2.7 g/plant for the control. However, the other nitrogen treatments gave no significant increase in top dry matter over the control plants.

TABLE 3: Interaction of inoculation by nitrogen in top dry matter yield of chickpea, 75 days after sowing.

Nitrogen treatment	Top DM yield (g/plant)	
	Nil	Inoculated
0 kg/ha	1.3	2.7
30 kg/ha at sowing	1.9	2.3
15 kg/ha at sowing plus 14 kg/ha 30 days after sowing	2.2	2.8
30 kg/ha 30 days after sowing	2.2	3.5
S.E. (Mean)	0.13	

TABLE 4: Effect of inoculation and nitrogen fertilizer application on root dry matter yield of chickpea, 60 and 75 days after sowing (DAS).

Treatment	Root DM yield (g/plant)	
	60 DAS	75 DAS
Inoculation (I)		
Nil	1.27	1.21
Inoculated (40 kg/ha)	1.44	1.16
L.S.D. (5%)	0.236	0.202
Nitrogen (N)		
0 kg/ha	1.18 b	1.06 b
30 kg/ha at sowing	1.31 b	1.08 b
15 kg/ha at sowing plus 15 kg/ha 30 days after sowing	1.33 b	1.30 a
30 kg/ha 30 days after sowing	1.62 a	1.30 a
I x N	NS	NS
S.E. (Mean)	0.056	0.049
C.V. (%)	11.8	11.6

Root dry matter was not affected by inoculation. However, nitrogen application increased root dry matter yield especially at 60 and 75 days after sowing. At the latter harvest date the magnitude of the nitrogen response was similar to that measured for the tops (Table 4).

When the ratio of plant tops to roots on a dry weight basis was calculated, no significant response to nitrogen treatments was observed. However, inoculation increased the top:root (T/R) ratio by 35% at 75 days after sowing. The T/R ratio was 2.45 in inoculated plants compared to 1.58 in un-inoculated plants. Again, there was a highly significant ($P \leqslant 0.01$ nitrogen by inoculation interaction at final harvest. Un-inoculated plants showed an increased T/R ratio when nitrogen was applied, but with inoculation the T/R value of the control did not differ from that of plants treated with 30 kg N/ha at sowing and 30 kg N/ha 30 days after sowing.

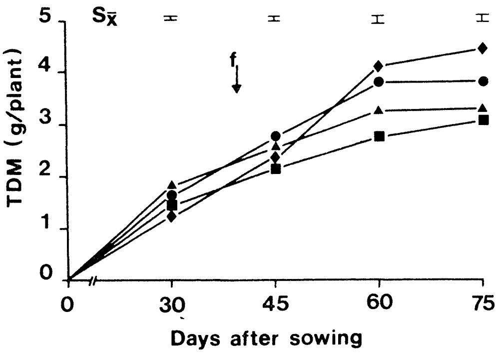

Figure 1: Changes in total dry matter in *Cicer arietinum* as affected by nitrogen fertilizer (■) Nil; (▲) 30 kg/ha at sowing; (●) 15 kg/ha at sowing plus 15 kg/ha 30 days after sowing; (◆) 30 kg/ha 30 days after sowing f = flowering.

Fig. 1 shows the changes in total plant dry matter accumulation as affected by fertilizer nitrogen application. Small and insignificant differences were observed during the early stages of growth but by 60 and 75 days after sowing, responses were significant. At 75 days after sowing, plants which had received 30 kg N/ha 30 days after sowing weighted 4.15 g while the split application of 15 kg N/ha at sowing and 15 kg N/ha 30 days after sowing had produced 3.79 g/plant. These values were significantly higher than the 3.05 g/plant from the control plants and 3.17 g/plant in plants given 30 kg N/ha at sowing. Inoculation increased total dry matter to 3.95 g/plant compared with 3.13 g/plant from un-inoculated plants, that is, a 20% increase at 75 days after sowing. There was a highly significant nitrogen by inoculation interaction for total dry matter yield per plant. The responses were similar to those obtained for top dry matter yield. There was a response to inoculation alone and in combination with nitrogen.

DISCUSSION

In this experiment, inoculation produced good nodulation of chickpea plants confirming the earlier findings of Corbin *et al.* (1977) that strain CC1192 is a suitable inoculant for this highly *Rhizobium* specific legume. The depression in the number of nodules formed during the vegetative stage (Table 1) when nitrogen was applied at sowing was consistent with reports for other legume crops (Pate and Dart, 1961; Kang, 1975; Ball *et al.*, 1983). However, from commencement of flowering to pod fill no significant effect of applied nitrogen was detected in total nodule number per plant. This suggests that the inhibition of nodulation by nitrogen occurred only at an early stage. The inhibition mechanism has not yet been fully identified but some workers suggest it is caused by a reduction of root hair production and curling (Munns, 1977) or the number of infection sites (Pate and Dart, 1961).

Inoculation alone and in combination with nitrogen increased total dry matter production. This was expected because of the zero-N fertility of the growth medium used. Addition of small quantities of nitrogen appeared to be fully warranted in this situation because plants which received no nitrogen, even when nodulated, were stunted during their early growth. In fields with low soil nitrogen fertility status, the importance of adding inorganic nitrogen during the early growth of well nodulated chickpeas has also been reported by Chundawat *et al.* (1976).

Late application of nitrogen seemed to contribute significantly to the nitrogen needs of the crop at later growth stages even when plants were nodulated. This was reflected in the increased total dry matter yield of the inoculated plants treated with 30 kg N/ha 30 days after sowing (Fig. 1). Earlier work (Allos and Bartholomew, 1959) had revealed that fixation processes never supplied sufficient nitrogen for maximum growth of legumes. With soybeans and other small seeded legumes they estimated that only about one-half to three-quarters of the total nitrogen for maximum yields is generally supplied by the fixation process. Dart *et al.* (1975), using chickpea grown in controlled environment found lower rates of nitrogen fixation in maturing plants. Chickpea fruits develop as strong sinks for carbon and nitrogen (Evans, 1982) and, as in soybean (Lawn and Brun, 1974), may require additional mineral nitrogen to meet the plant's total nitrogen demand. In this study, application of nitrogen fertilizer during the vegetative stage possibly contributed to increased total dry matter production.

This experiment showed an important interaction between inoculation and nitrogen fertilizer application by demonstrating a potentially detrimental effect of nitrogen applied at planting on symbiosis, whereas application within the growing period may allow the chickpea plant to utilize nitrogen from the soil and from symbiosis. Thus, it can be concluded from this work that inoculation combined with application of 30 kg N/ha 30 days after sowing might significantly increase total dry matter yield in chickpeas.

However, further studies are needed to determine if there is a critical stage of plant development when nitrogen is required to increase yield. Application of nitrogen at different levels and at various growth stages would indicate where efforts are needed to obtain most benefit from combined nitrogen.

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