

Rhizobium and nitrogen effects on chickpeas sown on two dates in Canterbury

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Abstract

Kabuli chickpeas (*Cicer arietinum* L.) were sown on 3 July and 30 September 1992 into a Templeton silt loam soil. Nitrogen at 0, 15, 45 or 90 kg/ha and *Rhizobium* inoculation at 0, 1 or 2 times the recommended rate were applied in a factorial design. Maximum dry matter (DM) yield which averaged 10.35 t/ha was not influenced by either *Rhizobium* inoculation or nitrogen rate. Early in the growing season (mid November) 90 kg N/ha had reduced nodule number by 50%, but nodule numbers did not differ at later assessments. However nodulation was poor and nodule number did not exceed 3 per plant. Additional N increased leaf area index (LAI) from 2.7 at 0 kg N/ha to 3.7 at 90 kg N/ha and total intercepted photosynthetically active radiation (PAR) from 725 MJ/m² to 760 MJ/m² at 0 and 90 kg N/ha respectively. Neither inoculation or nitrogen affected seed yield, which averaged 2.87 t/ha with a harvest index (HI) of 0.40. Harvest index was reduced by nitrogen application. The results suggest that if chickpeas are grown in a soil of moderate to high fertility neither additional nitrogen nor *Rhizobium* inoculation are necessary for maximum DM yield.

Additional key words: Chickpea, *Cicer arietinum*, nitrogen, inoculation, *Rhizobium*, sowing date.

Introduction

Many grain legumes derive most of their nitrogen (N) requirements from biological nitrogen fixation (Saxena, 1988), a source of N which is both cheap and conservative of soil mineral nitrogen (Evans, 1982). Extensive research suggests that chickpeas do not fix sufficient N for maximum growth (Rupela and Dart 1980; Hernandez and Hill, 1984). Mobilization of vegetatively stored N occurs during seed filling (Evans, 1982). Additional N fertilizer is therefore occasionally provided to stimulate nitrogen fixation and seed yield (Chopra-Khana and Sinha, 1987). Mineral N in the rhizosphere however, limits symbiotic nitrogen fixation as shown by decreased nodule numbers (Wong, 1980), nodule mass (Summerfield *et al.*, 1977) and decreased nitrogenase activity per unit mass (Streeter, 1981).

Grain legumes are more likely to be exposed to nitrate (NO₃⁻) than other N forms (Streeter, 1988) because transformations of applied urea and ammonium usually yields NO₃⁻ (Murphy *et al.*, 1986). If chickpea is to become a useful crop in the rotation, it must be able to enhance soil nitrogen under a range of soil inorganic nitrogen levels (Jessop *et al.*, 1984). There is therefore a need to define the level of inorganic nitrogen which

could enhance plant growth without suppressing nodulation and nitrogen fixation.

The work reported here was conducted to investigate the effect of sowing date, mineral N and inoculation on the nodulation, leaf area development, solar radiation interception, DM accumulation and yield of chickpeas in the Canterbury environment.

Materials and Methods

The experiment was carried out on a Templeton silt loam soil at the Lincoln University Henley Research Farm. A Ministry of Agriculture and Fisheries soil quick test gave the following results: pH 6.1, Ca 14, K 16, P 26, Mg 24, Na 4, S 2.

The experimental design was a split plot with two sowing dates, 3 July and 30 September 1992, as main plots and a factorial combination of 4 rates of nitrogen 0, 15, 45 or 90 kg/ha (applied as calcium ammonium nitrate) and 3 rates of *Rhizobium* inoculant 0, recommended rate (240 g/100 kg seed) or 2 x recommended rate in the sub-plots. There were three replicates and each plot was 21 m².

Locally obtained Kabuli chickpeas with a 1,000 seed weight of 450 g and a germination of approximately 60%

were used. Seed was treated with the fungicide Apron (ai metalaxyl 350 g/kg and captan 350 g/kg) at a rate of 200 g (dissolved in 500 ml of water) per 100 kg seed.

Rhizobium ciceri, strain CC1192 was obtained from Coated Seed Limited, Christchurch. The required amount of inoculum was mixed with 100 ml of water to form a thick slurry and then mixed thoroughly with the chickpea seed. Calcium ammonium nitrate (27% N) at the required rate was broadcast evenly on to the plots to be fertilized before drilling. The seed was drilled on the two sowing dates using an Öyjord cone seeder to give a plant population of about 45 plants/m².

Weed control was achieved by a pre-sowing application of cyanazine at 1.7 kg/ha in 320 l of water/ha. A further pre-emergence application of the same herbicide at the same rate was applied seven days after drilling. Further weed control was by hand.

Growth was followed by estimating above ground DM fortnightly from 0.2 m² cuts selected at random from each plot. The harvested samples were oven dried to constant weight and their dry weight recorded. Three plants, selected at random from the 0 and 90 kg N/ha plots, had their shoots cut off and their roots were recovered for nodule counts.

Solar radiation intercepted was measured using two miniature tube solarimeters, model TSM (DELTA-T Devices, Cambridge, England). Solarimeter output was integrated for 20 seconds using a two-channel integrator (Systel Engineering Ltd, Christchurch, NZ). A relative sensitivity check was done for 30 seconds at the start of the measurements and after every six plots. Leaf area

index was measured using a plant canopy analyzer (LICOR LAI 2000). At the final harvest total DM and seed yield (12% moisture) was estimated from 2 m² cuts taken from the central part of each plot.

Results

Nodulation

Average soil temperatures over the early growing season were low (Table 1) and nodulation was severely restricted. The rising temperature in November did little to improve the situation and nodule numbers averaged about 1 nodule per plant (Table 2) in both sowings in mid-November when the first nodule count was taken.

The addition of 90 kg N/ha reduced nodule numbers by 50% early in the growing season. However, as the growing season progressed, nodule numbers increased and at the final nodule count both treatments had the same number of nodules. Inoculation significantly ($p < 0.05$) affected nodulation at all sampling dates. However, nodules were recovered from plants from some uninoculated plots.

At the 27 January 1993 nodule harvest there was a significant ($p < 0.05$) but very small inoculation by sowing date interaction. Plants in the July sowing had 2.16 nodules per plant in the uninoculated control plots while the inoculated plants had 2.33 nodules per plant. On the same day plants from the September sowing had only 0.50 nodules per plant in the absence of inoculum but 3.00 nodules/plant in the inoculated plots.

Table 1. Meteorological data reported from Broadfield meteorological station in Lincoln, Canterbury during the 1992/93 season with the long term means¹ in parentheses.

| Month | Max. daily temp. (°C) | Min. daily temp. (°C) | Mean daily temp. (°C) | Mean soil temp. (°C) (10 cm) | Monthly total rainfall (mm) |
|----------|-----------------------|-----------------------|-----------------------|------------------------------|-----------------------------|
| 1992 Jul | 11.3 (10.1) | 2.5 (1.4) | 6.9 (5.7) | 2.2 (4.0) | 63.0 (68) |
| Aug | 9.8 (11.4) | 2.2 (2.7) | 6.0 (6.7) | 3.2 (5.2) | 166.8 (62) |
| Sept | 10.6 (14.2) | 3.3 (4.6) | 7.0 (9.4) | 4.6 (7.6) | 74.2 (47) |
| Oct | 14.5 (16.8) | 6.6 (6.7) | 10.6 (11.7) | 8.0 (10.8) | 81.2 (49) |
| Nov | 18.5 (18.8) | 9.2 (8.1) | 13.8 (13.6) | 11.6 (13.5) | 45.8 (53) |
| Dec | 18.2 (20.4) | 9.6 (10.4) | 13.9 (15.4) | 12.8 (16.0) | 55.4 (57) |
| 1993 Jan | 21.0 (21.3) | 10.0 (11.5) | 15.5 (16.4) | 13.6 (17.3) | 61.4 (60) |
| Feb | 20.2 (20.9) | 10.1 (11.4) | 15.2 (16.2) | 13.4 (16.5) | 44.0 (54) |

¹ Dates for long term means: Rainfall, 1930-1981; mean daily maximum, minimum and mean temperatures, 1975-1983; soil temperature (10 cm), 1976-1986.

Leaf area

Early vegetative growth was limited as shown by the slow development of leaf area. It was not until 144 DAS that the July-sown plants attained a leaf area index of 1 (Table 3).

However, within the next 30 days, LAI increased dramatically. At this time, nitrogen significantly ($p < 0.001$) increased LAI. The plants which had received 90 kg N/ha had an average LAI of 3.7 compared with 2.7 in the plants given no additional N. Towards the end of the growing season leaf senescence was slowed by N application (Table 3).

Throughout the growing season inoculation had no significant effect on LAI, although plants inoculated with the recommended rate of *Rhizobium* consistently had a higher LAI (Table 3).

Table 2. The effect of nitrogen application, *Rhizobium* inoculation and sowing date on nodulation of chickpea in Canterbury during the 1992/93 season.

| Treatment | Number of nodules/plant at each harvest date | | |
|------------------------------------|--|-----------|-----------|
| | 20 Nov 92 | 21 Dec 92 | 27 Jan 93 |
| Sowing date (S) | | | |
| July | 1.03 | 1.58 | 2.25 |
| September | 1.14 | 1.44 | 1.75 |
| SEM | 0.21 | 0.27 | 0.21 |
| Significance ¹ | NS | NS | NS |
| Nitrogen (kg N/ha) | | | |
| 0 | 1.42 | 1.61 | 2.00 |
| 90 | 0.75 | 1.41 | 2.00 |
| SEM | 0.21 | 0.27 | 0.21 |
| Significance | * | NS | NS |
| Inoculation² (I) | | | |
| None | 0.72 | 1.03 | 1.33 |
| Double rate | 1.44 | 2.00 | 2.66 |
| SEM | 0.21 | 0.27 | 0.21 |
| Significance | * | * | * |
| CV % | 67 | 61 | 37 |
| Interactions ³ | none | none | I x S |

¹ NS, not significant; *, $P < 0.05$

² Inoculation relative to recommended rate.

³ Significant treatment interactions at 5% level.

Radiation absorption

Photosynthetically active radiation intercepted by the chickpea canopy increased as leaf area increased, reaching a maximum of 9.3 MJ PAR/m² per day at the time maximum LAI was recorded. It then declined progressively as the growing season advanced.

Photosynthetically active radiation intercepted over the entire growing period averaged 743 MJ PAR/m². The July-sown crop intercepted more PAR at 810 MJ/m² compared with the 675 MJ/m² intercepted by the September-sown crop (Table 4). There was a significant ($p < 0.05$) linear increase in the total amount of PAR intercepted with increased N application.

Dry matter accumulation

Over the growing season DM accumulation followed a similar trend to the PAR intercepted. There was an

Table 3. The effect of nitrogen application, *Rhizobium* inoculation and sowing date on leaf area index of chickpea in Canterbury during the 1992/93 season.

| Treatment | Leaf area on harvest date | | |
|------------------------------------|---------------------------|----------|---------|
| | 24/11/92 | 24/12/92 | 26/1/93 |
| Nitrogen (kg N/ha) | | | |
| 0 | 0.66 | 2.72 | 2.18 |
| 15 | 0.70 | 3.04 | 2.39 |
| 45 | 0.71 | 3.15 | 2.34 |
| 90 | 0.72 | 3.70 | 2.72 |
| SEM | 0.06 | 0.13 | 0.01 |
| Significance ¹ | NS | ** | * |
| Inoculation² (I) | | | |
| None | 0.71 | 3.14 | 2.32 |
| Single rate | 0.74 | 3.22 | 2.57 |
| Double rate | 0.64 | 3.10 | 2.34 |
| SEM | 0.06 | 0.11 | 0.08 |
| Significance | NS | NS | NS |
| Sowing date (S) | | | |
| July | 1.04 | 3.26 | 2.32 |
| September | 0.35 | 3.04 | 2.49 |
| SEM | 0.05 | 0.09 | 0.07 |
| Significance | ** | NS | NS |
| Interactions³ | | | |
| Interactions ³ | none | none | none |
| CV % | 38.5 | 17.5 | 17.1 |

¹ NS, not significant; *, $P < 0.05$; **, $P < 0.01$

² Inoculation relative to recommended rate.

³ Significant treatment interactions at 5% level.

initial period when DM accumulation was very slow, then a log phase when DM increased at an increasing rate and a final phase when DM decreased at an increasing rate.

The maximum mean DM recorded was 10.35 t/ha and neither N application nor inoculation significantly affected DM accumulation. The July sowing produced significantly ($p < 0.001$) more maximum DM (11.19 t/ha) than the September sowing (9.51 t/ha) (Table 4).

Seed yield and harvest index

At the final harvest, seed yield averaged 2.87 t/ha. The July sowing yielded less (2.70 t/ha) than the September crop (3.03 t/ha) but the difference was not significant (Table 4). Similarly neither inoculation nor nitrogen had any effect on seed yield. However, HI was significantly ($p < 0.05$) reduced by nitrogen application (Table 4).

Discussion

Nodulation

Chickpea yield responses to inoculation have been variable. Hernandez and Hill (1983) obtained an average seed yield increase of 29% following inoculation. Their results compare favourably with an average seed yield increase of 27% reported by Cakmakci *et al.* (1988). In the present study seed yield was not increased by inoculation.

Nodulation and nodule development in legumes is influenced to a large extent by soil temperature and legumes nodulate slowly when soil temperatures are low (Corbin *et al.*, 1977). The results of this experiment provide further support of this observation. Nodulation in the non-inoculated control plots was observed and is an occurrence that has also been reported by other workers (Saxena and Yadav, 1975; Ibrahim and Salih,

Table 4. The effect of nitrogen application, *Rhizobium* inoculation and sowing date on total PAR intercepted, maximum DM accumulated, total dry matter, seed yield and Harvest Index in chickpea in Canterbury during the 1992/93 season.

| Treatment | PAR intercepted (MJ/m ²) | Maximum DM accumulated (t/ha) | TDM (t/ha) | Seed yield (t/ha) | Harvest Index |
|------------------------------------|--------------------------------------|-------------------------------|------------|-------------------|---------------|
| Nitrogen (kg N/ha) | | | | | |
| 0 | 725 | 10.21 | 6.65 | 2.80 | 0.42 |
| 15 | 736 | 9.63 | 7.06 | 2.92 | 0.40 |
| 45 | 750 | 10.49 | 6.97 | 2.74 | 0.39 |
| 90 | 760 | 11.06 | 7.44 | 3.01 | 0.40 |
| SEM | 12.40 | 0.50 | 0.25 | 0.11 | 0.01 |
| Significance ¹ | * | NS | NS | NS | * |
| Inoculation² (I) | | | | | |
| None | 736 | 11.01 | 6.68 | 2.68 | 0.40 |
| Single rate | 756 | 10.34 | 7.16 | 2.92 | 0.40 |
| Double rate | 736 | 9.69 | 7.26 | 2.99 | 0.41 |
| SEM | 10.70 | 0.44 | 0.22 | 0.09 | 0.01 |
| Significance | NS | NS | NS | NS | NS |
| Sowing date (S) | | | | | |
| July | 810 | 11.19 | 6.75 | 2.70 | 0.40 |
| September | 675 | 9.51 | 7.31 | 3.03 | 0.41 |
| SEM | 8.70 | 0.36 | 0.34 | 0.15 | 0.01 |
| Significance | ** | ** | NS | NS | NS |
| Interactions | NS | NS | NS | NS | NS |
| CV % | 7.1 | 20.7 | 15.1 | 15.7 | 6.7 |

¹ NS, not significant; *, $P < 0.05$; ** $P < 0.01$

² Inoculation relative to recommended rate.

1980). This may have been due to contamination from adjoining inoculated plots by water movement and during hand weeding.

Additional nitrogen inhibited nodule production only early in the growing season and this result is consistent with results reported in other chickpea experiments (Hernandez and Hill, 1984; Jessop *et al.*, 1984). This is attributed to increased soil temperature and the depletion of soil nitrogen as the season progressed.

Leaf area development

The maximum LAI of 3.2 recorded is less than the 3.5 and 3.7 reported by Hernandez and Hill (1985) and McKenzie *et al.* (1992) respectively. Throughout the growing season, inoculation had no effect on LAI (Table 3) but nitrogen increased it significantly. This increase was due to the initiation of more and broader leaves and a longer leaf area duration (LAD).

Radiation absorption

Development of leaf area and vertical growth in chickpeas is both slow and inherently limited. Canopy closure is therefore slow and solar radiation interception is less than maximum until canopy closure is complete (Chopra-Khana and Sinha, 1987). The total amount of PAR intercepted was significantly ($p < 0.05$) increased by nitrogen application and the 90 kg N/ha plots intercepted the most PAR. This was due to the higher maximum LAI attained and the longer LAD.

Dry matter accumulation

During their vegetative growth, crops accumulate DM at rates which are proportional to intercepted radiation (Monteith, 1977). Relationships between PAR intercepted and DM accumulation of this nature have been reported in chickpeas (Hughes *et al.*, 1987), wheat and barley (Gallagher and Biscoe, 1978), corn (Williams *et al.*, 1965) and soybean (Shibles and Weber, 1965). The results of this study show a similar trend and support Hernandez and Hill's (1985) finding that differences in DM production from chickpeas in Canterbury were related to the amount of solar radiation intercepted during the growing season.

Seed yield and harvest index

The mean seed yield of 2.87 t/ha obtained compares well with the 2.7 t/ha reported by Hernandez and Hill (1985) but is less than the 3.5 t/ha obtained by McKenzie *et al.* (1992), a consequence of the increased flower and pod abortion in the present study. Neither N application or inoculation increased seed yield, and HI was significantly reduced by N. The fertilized plots had

higher LAIs, intercepted more PAR and accumulated, on average, more DM. Most of this extra DM was not partitioned into seed and therefore HI was reduced by N application.

Conclusions

1. The addition of fertilizer N reduced the number of nodules developed early in the growing season.
2. The addition of N increased both LAI and PAR intercepted. However, it did not increase DM production or seed yield. The conversion of PAR into DM and the partitioning of the DM into seeds are factors limiting increased seed yield under these conditions.
3. Under conditions of moderate to high soil fertility neither additional N nor *Rhizobium* inoculation appear to be necessary to obtain maximum DM yield.

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