Australian Journal of Crop Science
1(1):11-22 (2008)

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Synergic effect of N and moisture on biochemical property of nodules and seed yield in chickpea

Gan YT [1*], Jayakumar P [1], Symons S [2], and McDonald CL [1]

[1]*Agriculture and Agri-Food Canada, Box 1030, Swift Current, SK, S9H 3X2, CANADA*
[2]*Grain Research Laboratory, 600-303 Main Street, Winnipeg, MB, R3C 3G8, CANADA*

[1*]*Corresponding author email:* gan@agr.gc.ca

Abstract

Water stress often has a negative effect on nodulation and seed yield in legumes but this effect can be minimized through N management. This study determined the synergic effect of water stress and N fertilization on the biochemical property of nodules, biomass partitioning among shoot, roots and nodules, and seed yield in chickpea. The cultivar 'CDC-Frontier' inoculated with and without *Mesorhizobium cicer* was grown at various rates of N fertilizer under high (90% field capacity), medium (60%) and low (30%) moisture levels in controlled environments. There was a significant synergic effect of N and moisture on nodulation and productivity. The seed yield of non-inoculated chickpea receiving zero N (0N) was reduced by 174% at high, 90% at medium, and 50% at low moisture levels compared to chickpea receiving 60 kg N ha^{-1}(60N). As soil moisture declined from high to low, the seed yield of inoculated chickpea supplied with 20 to 40N were reduced by 25%, significantly less than the yield loss of 58% for the plants supplied with 0N. Presence of N allowed a lower accumulation of amides in nodules and more N were kept in the nodules. The use of N fertilizer reduced the negative effect of water stress by partitioning more biomass to roots. Stronger root systems allowed plants to absorb more water for the transport of fixed N. In practice, yield losses from ineffective nodulation due to water stress can be minimized with the use of low doses of fertilizer N in chickpea.

***Keywords**: Cicer arietinum*; moisture stress; N_2 fixation; fertilizer N; Shoot:root ratio

Introduction

Chickpea (*Cicer arietinum* L.) is an important rain legume in the world with its total production of 7.1 MT, ranking third behind dry bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.) (FAO, 2004). This annual legume has been grown in semiarid regions of the world for hundreds of years (Siddique and Sykes, 1997; Kumar and Abbo, 2001). Inclusion of this N-fixer in cropping systems has shown to improve nutrient and water use efficiency (Miller et al., 2003), increase the yield and quality of subsequent crops (Gan et al., 2003a), and improve the economic sustainability of agricultural systems (Zentner et al., 2002). Being a legume, chickpea can form symbiotic association with rhizobia. Under favorable conditions, the symbiotic N_2 fixation can produce as high as 176 kg N ha^{-1} (Beck, 1992), and provide up to 85% of its N requirements (Rennie and Dubetz, 1986). Inoculation with an effective strain of *Rhizobium* is an economical way of enhancing seed yield and quality in chickpea (El Hadi and Elsheikh, 1999). However, rhizosphere colonization and nodule formation can be reduced substantially when soil moisture is low (Paul, 1998; Gan et al., 2005). Likewise, the proportion of N derived from symbiotic N_2 fixation is also decreased by water stress (Marcellos et al., 1998; Kurdali et al., 2002). Under drier conditions, chickpea can derive only a small proportion of crop N requirements from symbiotic N_2 fixation (Marcellos et al., 1998).
This is because soil moisture plays a critical role in both nodule formation and N_2 fixation. Low soil moisture during the early stages of the plant growth decreases nodule formation (Gan et al., 2005), and low moisture during late vegetative to early flowering period decreases efficiency of N_2 fixation

(Beck et al., 1991). Chickpea is usually grown as a post-rainy season crop (e.g., in the subtropics and Mediterranean) or a rain-fed crop (e.g., in North America). Thus, the crop is often subjected to terminal drought stress (Kurdali, 1996; Ali et al., 2002). An early onset of terminal drought can have a severe impact on the seed yield of chickpea relying on symbiotic N_2 fixation (Wery et al., 1988). In the case when the N_2 fixation activity is limited by terminal drought stress, chickpea plants may receive benefits from the use of low rates of fertilizer N where some synergy may be derived from the combination of N sources (Munns, 1977). The objective of this study was to evaluate the effects of different levels of fertilizer N, and different levels of water stress during reproductive development, on biomass partitioning between shoot, roots and nodules as well as on nodule efficiency and seed yield in chickpea. We hypothesized that a low dose of fertilizer N would benefit chickpea plants most when nodule function was negatively affected under water stress. We also hypothesized that the use of fertilizer N may alter biomass partitioning between roots and nodules, resulting in abundant root growth and great soil exploration for uptake of nutrients and water.

Materials and methods

Plants and growth conditions

Experiments were conducted at the Agriculture and Agri-Food Canada Semiarid Prairie Agricultural Research Centre, Swift Current, SK, Canada in 2005. The kabuli chickpea 'CDC Frontier', a popularly-grown cultivar in Canada, was used in this controlled environment growth chamber study. The rooting medium was prepared by mixing Swinton silt loam soil with sand and vermiculite at the proportion of 1:1:1 (v/v) using a soil mixer. The soil was collected from a field of the Research Centre on which no legumes had been grown in the past five years. The final rooting mixture contained 44% of soil, 51% of sand, and 5% of vermiculite by weight, and the amount of water held by the mix at field capacity (FC) was determined in the laboratory. Two-liter milk cartons (pots) were filled with 2.5 kg of the rooting mixture up to 3.5 cm from top with frequent tamping. Prior to pot filling, 3 holes were made at the bottom of the pot and 1 cm of commercial-grade peat moss was added to the bottom of the pots to prevent drainage of the rooting mixture. Triple super phosphate was added at the rate of 35 mg pot^{-1} (12 kg P ha^{-1}) at the time of potting. Prior to seeding, seed was treated with fungicides, to minimize soil- and seed-borne diseases. In each pot, three seeds were planted 30 mm deep with approximately 10 mm apart between each seed. The plants were thinned to one per pot at the 2- to 3-leaf stage. Plants were grown in a growth chamber (Model GR 96, CONVIRON, Control Environment Ltd., Winnipeg, MB, Canada) under a 22/16^{o}C day/night temperature regime and 16 h photoperiod. The temperature was maintained in a square wave fashion (i.e., rapid temperature transition). Relative humidity was set at 80% which was achieved through airflow introduced into the growth area through refrigerated coils. Built-in air handling plenums ensured a consistent air flow providing stable and uniform temperatures and RH in the chamber. Photosynthetic photon flux density (PPFD) was 494±12 µmol m^{-2} sec^{-1} (the mean of 10 readings made across the chamber bench where plants were grown). The PPFD readings were done near the canopy level (approximately 70 cm below the light bench) using an OL-2000Q quantum sensor (Optisk Laboratorium ATV, Lyngby, Denmark), and CO_2 in the chamber was not controlled or measured.

Experimental design

Two experiments were conducted. The first experiment consisted of 6 treatments where 3 moisture regimes and 2 N levels were arranged in a factorial (3 x 2), complete randomized block design with 9 pots treatment^{-1}. Plants were either not given any fertilizer N (0N), or supplied with fertilizer N at the rate of 60 kg ha^{-1} (60N). The fertilizer N was applied in the form of ammonium nitrate in solution at the time of planting. No *Rhizobium* was applied. During the vegetative growth period, soil moisture was brought to 95% of field capacity (FC) each morning by weighing individual pots. Beginning at flower bud stage (50 days after planting, DAP), the three soil moisture treatments were performed by maintaining pots at high (90% FC), medium (60% FC), and low (30% FC) levels, respectively. Water was withheld to allow the pots reach the weight corresponding to their target soil moisture content, and the moisture contents of the pots were adjusted daily. The second experiment consisted of 9 treatments that were arranged in a factorial (3 x 3), complete randomized block design with 9 pots treatment^{-1}. Three rates of N fertilizers at 0 kg N ha^{-1} (0N), 20 kg N ha^{-1} (20N), and 40 kg N ha^{-1} (40N) were tested at each of the 3 moisture levels: high (90% FC), medium (60% FC), and low (30% FC). Peat-based *Mesorhizobium cicer* inoculum (Nitragin Nitrastick GC®, Nitragin Inc., Brookfield, WI) was applied to the seed at the rate of 110 g 25 kg^{-1} of seed. The inoculant had >100 million viable cells of *M. cicer* g^{-1}. The growing conditions and the application of the water treatments in the second experiments were the same as those used in the first experiment. Both experiments were repeated twice over time in different growth chambers using different randomization plans. The second run of the experiments was initiated one month after the first run. All nodulation data were collected at early flowering (i.e., 71 DAP) in the experiment, because nodule formation in chickpea can last several weeks from the initiation of nodules (Khatsing and Ghonsikar, 1981) and N_2 fixation usually peaks during early flowering (Beck et al., 1991).

Nodule efficiency, nodule number and biomass production

Biomass partitioning, nodule number and nodule efficiency were measured on 3 plants treatment^{-1} at 71 DAP. Pots were soaked in water for 10 min to loosen the soil, and the plants were quickly removed with minimum disturbance. Dinitrogen fixation was determined by the classical acetylene reduction assay in which nodulated roots were placed in jars with 10% (v/v) acetylene (Turner and Gibson, 1980). Gas samples were taken after 60 minutes and analyzed for ethylene concentration using a Varian Star 3600 CX gas chromatograph fitted with a Porapak N column. The root and nodules were separated at the end of the assay, and the number of nodules per plant was counted and recorded. The shoot, root and nodule samples were dried separately in an oven with air circulation at 50°C for a minimum of 5 d and dry weights were recorded. The shoot to root ratio was calculated. The ethylene produced h^{-1} g^{-1} of nodule dry weight was calculated from ethylene produced h^{-1} plant^{-1}, and expressed as nodule efficiency.

Biochemical analysis

The soluble carbohydrate, ureide and amide contents were estimated on nodules collected from 3 plants treatment^{-1} at 71 DAP. The nodules were separated from the roots, freeze-dried, and stored at -80°C. Ethanol-soluble carbohydrates were extracted from 25 mg of freeze-dried nodules following grinding in 1 ml of 80% ethanol at 23°C (Gallagher et al., 1997). The extracts were incubated for 10 min, centrifuged for 5 min (10,000 *G*) at room temperature (23°C) and the supernatant retained. This extraction was repeated twice, the supernatants were pooled and adjusted to 5 ml. Total sugar was estimated by Anthrone method (Yemm and Willis, 1954). Amino compounds were extracted from a 50 mg sub-sample of nodules by boiling in 1 ml of 0.2 M NaOH for 30 min (Vadez et al., 2000). Samples were centrifuged (10,000 *G*) at room temperature, refrigerated overnight and 100 µl of supernatant was used for measurement. Total amino compounds were determined spectrophotometrically using ninhydrin (Moore and Stain, 1948) with aspartate (Asp) as the standard. Ureide extraction was prepared identically to the extraction of amides, except that it was done on a 100 mg sub-sample. Samples were centrifuged and 0.1 to 0.2 ml of supernatant was used for ureide determination. Ureide was determined by basic hydrolysis of allantoin to allantoic acid, acid hydrolysis of allantoic acid to glyoxylate and urea, and spectrophotometric determination of glyoxylate after its reaction with phenylhydrazine and ferric cyanide (Vogels and Van der Drift, 1970).

Yield and yield components

Three plants per treatment were used for final yield analyses. Watering of plants was stopped 10 days before plants were harvest by hand at DAP 110. The number of pods plant^{-1} and the number of seeds pod^{-1} were determined. The seeds were air-dried and weighed, and seed yield recorded on a dry weight basis. The seed samples were sent to the Grain Research Laboratory, Canadian Grain Commission, Winnipeg, Manitoba for seed size measurement using image analysis.

Statistical analysis

The statistical analyses to determine the individual and interactive effects of N fertilization and moisture stress were conducted using JMP 5.0.1.2 (SAS Institute Inc., 2002). Statistical significance was declared at $P \leq 0.05$ and $P \leq 0.01$. Treatment effects from the two runs of experiments followed a similar trend, and thus the data from the two independent runs were combined in the analysis.

Results

No *Rhizobium* was applied to the plants in experiment 1, whereas in experiment 2, the plants were inoculated with peat-based *Mesorhizobium cicer Rhizobium* at the rate of 110 g 25 kg^{-1} of seed, with >100 million viable cells of *M. cicer* g^{-1}. Therefore, the results were presented separately for each of the experiments to show clearly the

Table 1. **Biomass partitioning of non-inoculated chickpea plants grown under various levels of soil moisture and N fertilizer**

Treatment effect	Biomass partitioning				
	Shoot	Root	Nodules	Underground	Shoot::root ratio
	------------------------------------ g $plant^{-1}$ ------------------------------------				
Soil moisture (% FC)					
High (90)	8.4	4.7	0.24	4.9	1.9
Medium (60)	7.8	5.0	0.24	5.2	1.7
Low (30)	6.7	5.0	0.10	5.1	1.4
Mean	7.6	4.9	0.20	5.1	1.7
Significance	**	ns	ns	ns	*
N fertilizer (kg ha^{-1})					
0	5.3	3.4	0.18	3.6	1.7
60	10.0	6.3	0.21	6.5	1.6
Mean	7.6	4.9	0.20	5.1	1.7
Significance	**	**	ns	**	ns

FC denotes field capacity.
*, ** and ns denote the difference at $P \leq 0.05$, $P \leq 0.01$, and no significance, respectively.

effect under each of the two inoculation conditions. Nodule formation and functionality were only determined for the inoculated plants in experiment 2.

Biomass and shoot to root ratio

Experiment 1

Nitrogen fertilization and moisture availability had a significant effect on the shoot dry matter (DM) production of chickpea (Table 1). Increasing moisture increased the shoot DM significantly, but it did not affect root DM. Application of N fertilizer had a significant effect on both shoot DM and root DM. Plants grown at 60N had 83% greater root DM than plants grown at 0N. The nodule production of these plants was marginal because the plants were not inoculated. Chickpea plants grown under low moisture conditions had a lower shoot to root ratio (1.4) than plants grown under high moisture (1.9). Nitrogen application, either individually or in combination with moisture, did not affect shoot to root ratio.

Experiment 2

Fertilizer N did not affect the shoot DM of inoculated chickpea, whereas the moisture effect on shoot DM was significant (Table 2). Similarly, moisture had a significant effect on the overall underground biomass production of the inoculated chickpea plants, and the underground DM decreased with decrease in soil moisture. Although fertilizer N did not affect the overall underground production, it had an effect on biomass partitioning between root and nodules. Plants inoculated with *Rhizobium* in the presence of 40N allocated 93% of the underground biomass to root production, significantly greater than those grown at 20N (76%) and 0N (64%). On the contrary, plants grown at 0N allocated 37% biomass to nodule growth, significantly greater compared to 23% at 20N and 7% at 40N. Moisture did not have any effect on root DM, but it had a significant effect on the nodule production. Nodule biomass accumulation decreased with the decline of soil moisture. The inoculated chickpea in the presence of fertilizer N (20N and 40N) had lower shoot to root ratio compared to plants at 0N, across the different levels of moisture. Decrease in soil moisture from high to low lowered the shoot to root ratio.

Nodule formation and functionality

Fertilizer N had a significant, negative effect on the number of nodules formed on the chickpea roots in experiment 2 (Table 3). On average, application of 20N decreased the number of nodules by 12%, and application of 40N decreased by 48% compared to the 0N control. Moisture and fertilizer N had interactive effects on nodule efficiency. Under high

Table 2. **Biomass partitioning of inoculated chickpea plants grown under various levels of soil moisture and N fertilizer**

Treatment effect	Biomass partitioning				
	Shoot	Root	Nodules	Underground	Shoot::root ratio
	g $plant^{-1}$				
Soil moisture (% FC)					
High (90)	14.7	6.8	2.1	9.0	2.4
Medium (60)	11.4	6.1	1.8	7.9	2.1
Low (30)	8.2	6.1	1.4	7.4	1.5
Mean	11.5	6.3	1.7	8.1	2.0
Significance	**	ns	**	*	**
N fertilizer (kg ha^{-1})					
0	10.9	4.7	2.7	7.5	2.5
20	11.8	6.4	1.9	8.4	1.9
40	11.7	7.9	0.6	8.4	1.5
Mean	11.5	6.3	1.7	8.1	2.0
Significance	ns	**	**	ns	**

FC denotes field capacity.
*, ** and ns denote difference at $P \leq 0.05$, $P \leq 0.01$, and no significance, respectively.

moisture conditions, 20N decreased the nodule efficiency by 22% as compared to the 0N control, whereas at 40N nodule efficiency decreased by 65%. Under medium moisture conditions, the nodule efficiency of the plants at 40N was greatly reduced compared to 0N and 20N. Under low moisture conditions, the nodule efficiency was near zero. Similarly, fertilizer N and soil moisture had significant effects on nodule soluble carbohydrate level (Table 3). Moisture had a detrimental effect on the accumulation of amides in nodules. The amide content of the nodules increased with the decrease in soil moisture. Nodules produced by plants at 0N showed a large increase in the amide content at medium moisture compared to plants grown at 20N and 40N. The amount of ureides was marginal in the nodules of all plants.

Pod formation and seed yield

Experiment 1
Nitrogen and moisture had interactive effects on the number of pods, pod fertility, and seed yield of non-inoculated chickpea plants (Table 4). At 0N control, moisture stress did not have any effect on the proportion of fertile pods (i.e., pods with at least one seed), whereas in plants grown at 60N, the proportion of fertile pods decreased from 86% at high to 70% at medium and further to 33% at low moisture level. At high and medium moisture levels, plants that received 60N produced more fertile pods compared to the 0N control (Fig. 1a). Application of 60N also increased the seed yield significantly at all levels of moisture compared to 0N control (Fig. 1b). The magnitude of the yield increase with N fertilization decreased with decreasing moisture; plants that received 60N increased the seed yield by 174% at high, by 90% at medium and by 50% at low moisture compared to the control plant which did not receive N fertilization.

Experiment 2
In chickpea that was inoculated with *Rhizobium*, moisture had a significant effect on pod production when coupled with fertilizer N (Table 4). At medium moisture, plants that received 0N and 20N produced fewer pods than plants that received 40N. At high and low moisture conditions, plants produced similar number of pods regardless of fertilizer N. Moisture and fertilizer N had a significant, interactive effect on the pod fertility of chickpea. When compared to the 0N control, the presence of 20N and 40N did not increase the proportion of fertile pods at high moisture, but they substantially increased the proportion of fertile pods at medium and low moisture levels. In plants that received 0N, the proportion of fertile pods decreased from 63% at high to 35% at medium moisture (28 percentage points of reduction) (Fig. 2b). Similarly, there was a reduction in the proportion of fertile pods in plants that received 20

***Table 3. N*odule formation and functionality of inoculated chickpea plants grown under various levels of soil moisture and N fertilizer**

N fertilizer (kg ha^{-1})	Soil moisture (% FC)	Nodule number	Nodule Efficiency [a]	Nodule soluble sugars [b]	Nodule amides [b]
0	High (90)	216	2.7	44	23
	Medium (60)	226	1.4	30	30
	Low (30)	210	0.0	17	39
20	High (90)	196	2.2	28	20
	Medium (60)	200	1.5	24	23
	Low (30)	179	0.0	14	36
40	High (90)	147	0.9	14	23
	Medium (60)	93	0.6	11	21
	Low (30)	101	0.0	15	26
N		**	**	**	ns
Moisture		ns	**	**	**
N * Moisture		ns	ns	**	ns

FC denotes field capacity.
[a]µmol C_2H_4 h^{-1} g^{-1} dry weight of nodules.
[b]mg g^{-1} dry weight of nodules.
*, ** and ns denote difference at $P \leq 0.05$, $P \leq 0.01$, and no significance, respectively.

N, but the magnitude of reduction was lower (21%). In plants that received 40N, the reduction was only 4%. Fertilizer N and moisture had a significant, interactive effect on seed yield of the inoculated chickpea (Table 4). At high moisture, plants inoculated with *Rhizobium* had similar seed yield regardless of fertilizer N. At medium moisture, the seed yield of plants that received 0N was decreased by 58%, whereas the seed yields of plants at 20N and 40N were decreased by 30% and 23%, respectively, from their yields obtained at high moisture (Fig. 2c). Neither N nor moisture had individual or interactive effect on the seed size (Table 4).

Discussion

Synergic effect of N and moisture

Fertilizer N had a positive effect on the pod fertility and seed yield of chickpea even the plant was inoculated with an effective *Rhizobium* strain. The magnitude of this effect was subjected to water stress during the reproductive development. When soil moisture changed from high (90% field capacity) to low (30% field capacity), the plants that received 0N decreased the proportion of fertile pods by 63%, whereas the plants that received 20 N decreased the pod fertility by 21%, and in plants that received 40N, the reduction of pod fertility was only 4%. Likewise, as soil moisture declined from high to low, the seed yield of the inoculated chickpea supplied with 20 to 40N were reduced by 25%, significantly less than the yield loss of 58% for the plants supplied with zero N. Our findings suggest that when nodulation and N_2 fixation are affected due to moisture stress, the inoculated chickpea plants require an alternate source of N in order to maintain pod fertility and seed yield. Under conditions with high moisture stress, chickpea plants are unable to perform an effective nodulation to produce N they require for continuous growth and development but it is still capable to uptake soil N that is supplied through fertilization.

Our study also showed that the negative effect of moisture stress on nodule efficiency was reduced when the plants were supplied with fertilizer N. Nodule efficiency of inoculated chickpea that received 0N was reduced by 70% when soil moisture level was decreased from high to medium, whereas the nodule efficiency of plants that received 20N was reduced only by 36%. This was

Table 4. **Pod and seed formation of chickpea with and without *Rhizobium* inoculant under various levels of soil moisture and N fertilizer**

N fertilizer (kg ha^{-1})	Soil moisture (% FC)	Number of pods at harvest	Proportion of fertile pods (%)	Seed yield (g $plant^{-1}$)	Seeds ≥ 9 mm In diameter (%)
Without *Rhizobium* inoculant (Experiment 1)					
0	High (90)	54	34	2.9	59
	Medium (60)	24	39	2.7	79
	Low (30)	17	39	1.8	71
60	High (90)	54	86	7.6	64
	Medium (60)	46	70	5.0	68
	Low (30)	16	33	2.4	63
	N	ns	**	**	ns
	Moisture	**	**	**	ns
	N * Moisture	**	*	**	ns
With *Rhizobium* inoculant (Experiment 2)					
0	High (90)	76	63	6.4	79
	Medium (60)	43	35	2.8	81
	Low (30)	25	27	1.9	90
20	High (90)	75	67	6.6	79
	Medium (60)	46	46	4.4	78
	Low (30)	27	29	2.1	87
40	High (90)	74	71	6.3	74
	Medium (60)	62	67	4.8	88
	Low (30)	20	34	2.7	71
	N	ns	**	ns	ns
	Moisture	**	**	**	ns
	N * Moisture	ns	ns	*	ns

FC denotes field capacity.
*, ** and ns denote difference at $P \leq 0.05$, $P \leq 0.01$, and no significance, respectively.

probably due to lower accumulation of amides in nodules in the presence of N, as less N is fixed leaving less N to be transported out of the nodules. Biomass partitioning data revealed that the use of fertilizer N reduced the amount of biomass invested in nodules, while it increased biomass investment in roots. As a result, inoculated plants that received fertilizer N had a greater root to shoot ratio compared to plants that did not receive N. This N effect on the root biomass was interacted with the availability of soil moisture. These results further suggest that chickpea plants supplied with fertilizer N have greater water use efficiency due to stronger rooting systems that allow the plants to extract more water from deep soil layers (Hudak and Patterson, 1995). In addition, stronger root systems allow plants to allocate more water for the transport of fixed N. Previous studies have also shown that residual nitrate in the soil affects biomass partitioning between roots and nodules (Jensen, 1986; Lodeiro et al., 2000). Plants relying exclusively on symbiotic N_2 fixation have less root biomass, but more nodule mass, than plants supplied with nitrate (Voisin et al., 2002).

Higher biomass investment in nodules is favorable for plant growth when water is available in adequate quantity, whereas higher investment in root tissues is essential for better soil exploration. Common bean genotypes that were more tolerant to drought had a superior rooting pattern than those

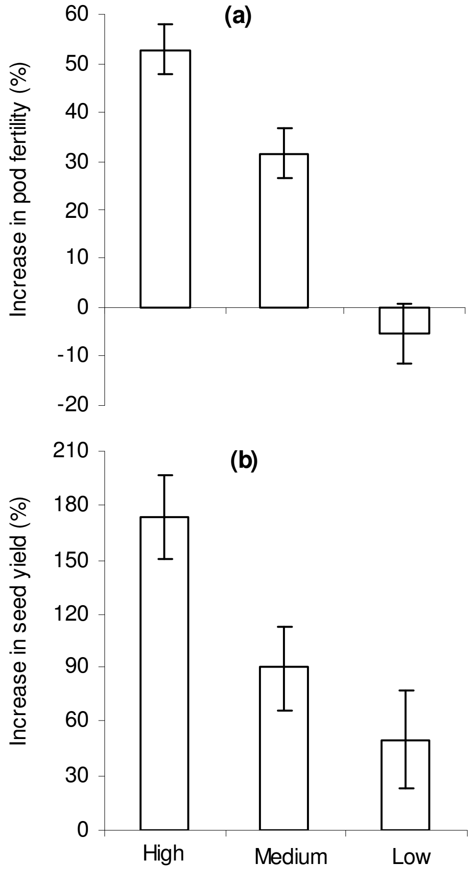

Fig 1. **Influence of N fertilization (60 kg ha^{-1}) on (a) pod fertility and (b) seed yield of non-inoculated chickpea at different levels of soil moisture, expressed as percentage increase when compared to plants that did not receive N fertilizer. The vertical bars indicate standard errors of means. High, medium and low denote 90%, 60% and 30% field capacity, respectively.**

that were less tolerant, due to stronger ability of the rooting system to extract more water from deep soil layers (Hudak and Patterson, 1995). Ali et al. (2002) reported that the more drought tolerant chickpea genotypes had greater root systems than the less tolerant genotypes.

Fertilizer N and Rhizobium

Application of fertilizer N at 60 kg N ha^{-1} (60N) nearly doubled the seed yield of non-inoculated chickpea compared to the plants that did not receive N. When N_2-fixing *Rhizobium* was applied, both biomass and seed yield of chickpea were greatly enhanced regardless of fertilizer N. Increased seed yield due to fertilizer N or N_2-fixation was mainly due to increased number of pods and increased percentage of fertile pods. Similar results have been reported previously by others (El Hadi and Elsheikh, 1999; Kyei-Boahen et al., 2002; Gan et al., 2005). Walley et al. (2005) reported that N application did not affect the seed yield of inoculated 'Sanford' kabuli chickpea, but application of N promoted early vegetative growth. This initial positive response of chickpea plants to N could be due to early N deficiencies after the seed N has been utilized but before the N_2-fixing association is fully established (Sprent and Minchin, 1983). In our study, N application to inoculated plants did not increase biomass production measured at the mid-flowering stage, suggesting that chickpea plant tissues developed early in the growing season may not be an effective N source for the later reproductive sink responsible for seed development. Fertilizer N may not be required for kabuli chickpea production when growing conditions are favorable for N_2 fixation, although it may increase early vegetative growth and tissue N concentrations.

It is unclear in the literature whether chickpea plants can translocate N from vegetative tissues to reproductive organs. Doughton et al. (1993) found that chickpea plants (cv. Reselected Tyson) that had a plentiful supply of soil mineral N accumulated significantly more plant N than plants dependant on N_2 fixation, but the seed yield was unaffected by the source or quantity of plant N accumulated. Bonfil and Pinthus (1995) reported that application of 100 kg N ha^{-1} at sowing promoted N uptake by the chickpea plants (cv. Bulgarit); however, final seed yield was not enhanced. Similarly, McConnell et al. (2002) reported that fertilizer N application resulted in higher shoot biomass and plant tissue N concentrations at anthesis for kabuli chickpea (cv. Dwelley), but the seed yield was not enhanced. In our study, presence of N negatively affected nodule formation and efficiency. On average, application of 20N to inoculated plants decreased the number of nodules by 12%, whereas application of 40N decreased the nodule number by 48% compared to 0N control. Similarly, application of 20N decreased the nodule efficiency by 22%, whereas 40N decreased the efficiency by 65% compared to the 0N control. Similar negative effects of N fertilizer on nodulation and N_2 fixation has been reported in studies for other legumes (Munns, 1977; Abaidoo et al., 1990; Doughton et al., 1993; Clayton et al., 2004; Walley et al., 2005). This leads to the conclusion that, when conditions are favorable for nodulation and N_2 fixation, application of fertilizer N will partly replace N from fixation without positively increasing productivity.

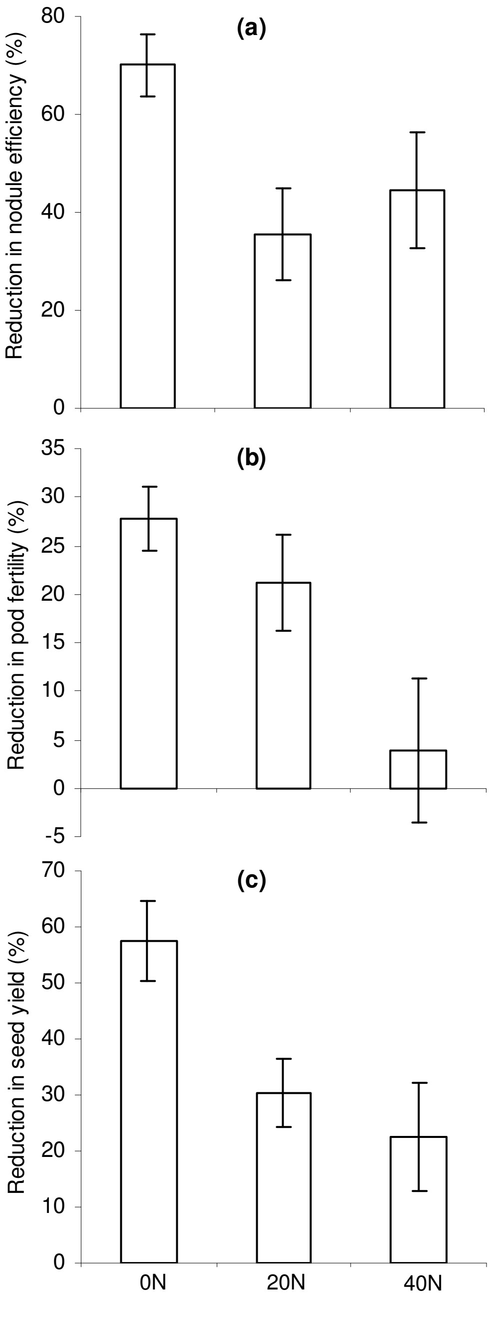

Fig 2. **Effects of decline in soil moisture from high (90% field capacity) to medium (60%) on (a) nodule efficiency, (b) pod fertility and (c) seed yield of inoculated chickpea supplied with different levels of N fertilizer (kg N ha^{-1}), expressed as percentage decrease at medium compared to high moisture level. The vertical bars indicate standard errors of means.**

Moisture and biochemical property of nodules

It is well known that soil moisture affect nodulation and N_2-fixation, but little is known about the relationship between moisture and biochemical properties of nodules in chickpea. In our study, chickpea grown under high (30% field capacity) to medium (60% field capacity) moisture stresses significantly decreased the N_2-fixing efficiency of the nodules and lowered carbohydrate supply to the nodules. Soil moisture also had a detrimental effect on the translocation of N from nodules to plant tissues in the form of amides. The amide content in the nodules was low under high soil moisture stress. Similarly, Sridhara et al. (1995) reported that effectiveness of nodules in soybean (*Glycine max* L.) decreased as a result of moisture stress either during flowering or pod filling. Reduction in nodule efficiency due to low moisture has also been reported by other researchers (Kumar and Pareek, 1984; de Vries et al., 1989; Marcellos et al., 1998; Kurdali et al., 2002). The reduction in nodule efficiency due to water stress might be attributable to higher allocation of photosynthates for reproductive development and N assimilation from the soil leaving the nodules with reduced assimilates for N_2 fixation. Also, lower rates of water movement out of the nodules during water stress might have restricted the export of products of N_2 fixation, thus inhibiting N_2 fixation via a feedback mechanism (Pate et al., 1969). Nitrogenous signal, associated with N accumulation in the shoot and nodule, exists in legume plants so that N_2 fixation is inhibited early when soils are dry (Serraj et al., 1999).

Moisture stress significantly reduced the seed yield of inoculated chickpea, and the yield loss was largely due to the reduction of pod production. In both experiments, high moisture stress significantly decreased the total number of pods per plant and the number of seeds per pod. Some earlier studies have also reported that moisture stress negatively affected seed yield in other legumes. Purcell and King (1996) reported that, under drought conditions, N and biomass accumulation and seed yield were lower for soybean plants dependent on N_2 fixation when compared to plants supplemented with fertilizer N. Dejong and Phillips (1982) reported that N accumulation by N_2-dependant clover (*Trifolium subterraneum* L.) was inhibited to a greater extent than nitrate-dependent plants due to water stress. Wery et al. (1988) showed that drought stress in chickpea depressed N_2 fixation more than plant growth and seed yield; mineral N supply stimulating plant growth under drought stress.

Conclusions

Fertilizer N had a positive effect on the pod fertility and seed yield of chickpea even the plant was inoculated with an effective *Rhizobium* strain. The magnitude of this effect was subjected to water stress during the reproductive development. As soil moisture declined from high to low, the yield loss for the chickpea supplied with low doses of N fertilizer was significantly less than the yield loss for the plants supplied with no N. Under conditions with high moisture stress, chickpea plants were unable to perform an effective nodulation to produce N required for continuous growth and development but it is still capable to uptake soil N supplied through fertilization. Moisture stress had a negative effect on nodulation, but this negative effect was reduced significantly when the plants were supplied with low doses of fertilizer N. Lower accumulation of amides in nodules in the presence of N resulted in less N to be transported out of the nodules. Biomass partitioning data revealed that the use of fertilizer N reduced the amount of biomass invested in nodules, but it increased biomass investment in roots. Stronger root systems allowed plants to allocate more water for the transport of fixed N to aboveground organs. In practice, application of low doses of fertilizer N increased pod fertility and seed yield of chickpea, particularly when the inoculated chickpea was under moisture stress during reproductive development. Although nodule numbers and their functionality were negatively affected by the application of inorganic N, greater seed yield with low doses of fertilizer N was achieved. By using low doses of fertilizer N, chickpea producers can minimize potential yield losses from inoculation failure due to unpredictable factors such as moisture stress.

Acknowledgements

We thank Dr. Chantal Hamel for reviewing the manuscript prior to submission. We also acknowledge the excellent technical assistance of Cal McDonald, Greg Ford, Ray Leshures, Lee Poppy, and Stefan Sigurdson, and the financial support of this project from Natural Sciences and Engineering Research Council of Canada, and Improving Farming System and Practices (IFSP) Program of Agriculture and Agri-Food Canada.

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