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PARTITIONING OF ^{14}C-PHOTOSYNTHATE IN LOW AND HIGH NODULATING SELECTIONS OF CHICKPEA

S.P. WANI, S. SIVARAMAKRISHNAN,
O.P. RUPELA, C. JOHANSEN, K.K. LEE
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT),
Patancheru, Andhra Pradesh,
India

Abstract

PARTITIONING OF ^{14}C-PHOTOSYNTHATE IN LOW AND HIGH NODULATING SELECTIONS OF CHICKPEA.
Genetic variation for nodulation capacity has been detected in chickpea cultivars. Five groups of cultivar ICC 5003, were selected based on increasing nodule number and mass. These groups were examined to understand carbon budgeting in relation to N_2 fixation. Chickpea plants grown at two mineral nitrogen levels were exposed to $^{14}CO_2$ and the distribution of ^{14}C-photosynthate in different plant parts was determined. Total plant biomass of chickpea selections increased significantly ($P < 0.01$) with increasing mineral N and nodulation level. Mean percentage ^{14}C-photosynthate translocated to the roots decreased with increasing nodulation rank and also with increasing mineral N level. Low nodulating selections of chickpea grown under low soil N level were unable to fulfil their N requirements through biological nitrogen fixation (BNF) as indicated by their higher root/shoot ratio compared to the high nodulating selections. Such plants invested more carbon in increased root production so as to exploit soil N as evidenced by the greater partitioning of ^{14}C-photosynthate to roots in low nodulating selections.

1. INTRODUCTION

Legumes have an important role in sustainable farming systems due to their on-farm nitrogen contributions through biological nitrogen fixation. Chickpea is one of the major grain legumes and can fix up to 141 kg N ha^{-1} season^{-1} [1]. Nitrogen fixation by legume-rhizobial symbioses uses products of photosynthesis, and therefore it competes for the photosynthate partitioned to economic yield [2-3]. Thus, carbon and energy budgeting of BNF by nodulated legumes continue to attract research interest [4-5]. Genetic variation for nodulation capacity has been detected within chickpea cultivars [6]. Five selection groups have been identified in a cultivar ICC 5003, S_1 to S_5, based on increasing order of nodule mass[6]. High nodulating selections (S_3 and S_5) of chickpea cultivar ICC 5003 grown under low available soil N conditions (10-12 mg N kg^{-1} soil) yielded 68% higher grain than the low nodulating selections (S_1 and S_2) grown under the same conditions [6]. We used these selections to study the relationship between N_2 fixation characteristics and ^{14}C-photosynthate translocation to different plant parts in order to understand carbon budgeting in relation to N_2 fixation in chickpea.

2. MATERIALS AND METHODS

2.1. Plants

Chickpea plants were grown in Vertisol (Typic Pellustert) in pots in a greenhouse at ICRISAT Center, Patancheru, India, during the 1991 postrainy season. The characteristics of the soil used in the pots were as follows: pH (1:2,soil:H_2O) = 8.2, Total N = 624 mg kg^{-1}, Olsen's P = 2.5 mg kg^{-1} and chickpea rhizobia = 10^3 g^{-1} soil.

2.2. Experimental design

Glucose at 1:6 proportion of available N (134 mg glucose in 298 g of soil) was applied in solution two weeks prior to sowing and also at 15 days after sowing (DAS) to immobilize available soil N and to create a low available N treatment (N1) of 14 ug N g^{-1} soil at sowing. Untreated soil served as the high available N treatment (N2) which contained 22 μg N g^{-1} soil, and received 1.5 mg N pot^{-1} (5 μg N g^{-1} soil) in solution prior to sowing. Single plants were grown in 7 cm square plastic pots filled with 298 g soil. At sowing, each pot received 10^5 rhizobia of strain IC 59 . Four selections with distinct nodulation ratings [S_1 and S_2 (low), S_3 and S_5 (high)] developed from ICC 5003 (=K 850), a released chickpea cultivar were used. Each treatment was replicated four times using a factorial randomized design. The plants were grown for 57 days in a greenhouse maintained at a day/night temperature regime of 26 ± 2 / 20 ± 2°C. Plants were watered to 70% water holding capacity with deionised water.

2.3. ^{14}C-Labeling of plants

Six days prior to harvesting, whole chickpea plants were exposed to $^{14}CO_2$. Plants were enclosed in a rectangular plexiglass chamber (39x28x28 cm, width x length x height) fitted with two small fans and an injection port rubber. Radioactive CO_2 was generated externally from ^{14}C-sodium bicarbonate ($NaH^{14}CO_3$) in a reservoir filled with HCl. $^{14}CO_2$ from the reservoir was filled in an air-tight glass syringe and 3 ml of $^{14}CO_2$ (about 40 μCi) was injected into the chamber through the injection port. Plants were exposed to $^{14}CO_2$ inside the chamber for 15 min and were harvested six days later.

2.4. Enzyme and isotope analysis

Nitrogenase activity in roots was assayed by measuring acetylene reduction activity immediately after harvest. Roots and nodules collected from the soil and plant samples were oven dried at 80°C for three days. Finely ground plant subsamples were used for determining ^{14}C-activity in different plant parts by oxidation in a Biological oxidizer (Model 300, R.J. Harvey Inc).

3. RESULTS AND DISCUSSION

Total plant biomass (shoots, roots, and nodules) of chickpea selections increased significantly ($P < 0.01$) from 1.5 g $plant^{-1}$ at N1 to 2.1 g $plant^{-1}$ at N2 treatment. Similarly, total plant biomass increased significantly ($P < 0.01$) with increasing nodulation level. A significant interaction between N level and nodulation rank of chickpea selections was also observed for total plant biomass produced; the increase in plant biomass with nodulation ranking at N1 was much greater than at N2 (Fig 1).

Root mass was similar across all N levels, and nodulation selections (Fig 2). Nodule numbers per plant were unaffected by increasing N level, however, nodule number increased significantly from 1.6 nodules $plant^{-1}$ in S_1 selection to 60 nodules $plant^{-1}$ in S_5 selection. The increase in nodule number resulted in a proportional increase in nodule mass per plant (Fig. 3). Nodule mass ($P < 0.01$) reduced in plants grown at the higher nitrogen level($P < 0.01$, Fig 3) with exception of the S_3 selection.

Total nitrogenase (C_2H_2 reduction) activity of plants grown at low soil N status was dependent on the degree of nodulation with the maximum activity observed in the S_5 selection (Table 1). Nitrogenase activity, however, was similar in all selections at the higher nitrogen level. Nitrogenase activity per unit root mass increased with the degree of nodulation selection (880 n mol

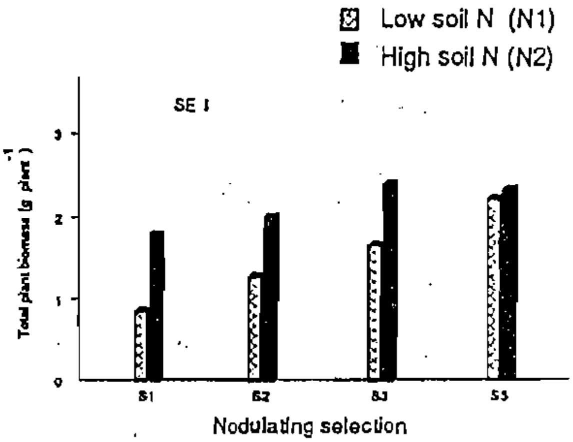

Figure 1. Total plant biomass (shoots, roots, and nodules) of different nodulating selections of chickpea cv ICC 5003 grown at two soil N levels in pots.

S1-S2 low-nodulating selection
S3-S5 high-nodulating selection

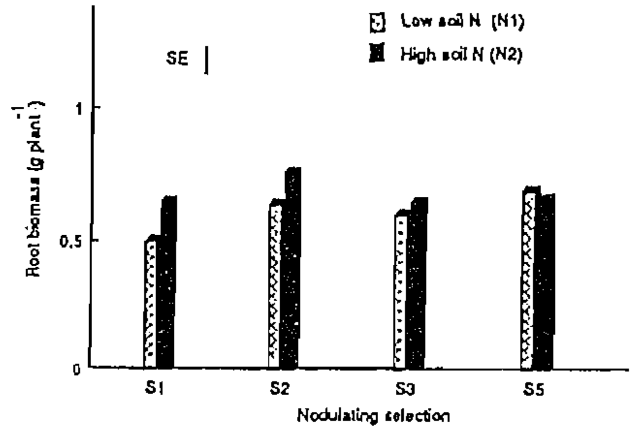

Figure 2. Root biomass of different nodulating selections of chickpea cv ICC 5003 grown at two soil N levels in pots.

S1-S2 low-nodulation selection
S3-S5 high-nodulation selection

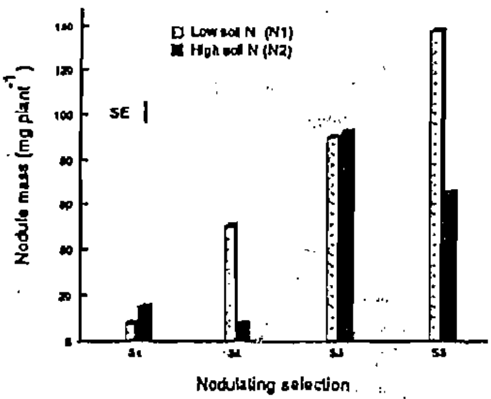

Figure 3. Nodule mass of different nodulating selections of chickpea cv ICC 5003 grown at two soil N levels in pots.

S1-S2 low-nodulating selection
S3-S5 high-nodulating selection

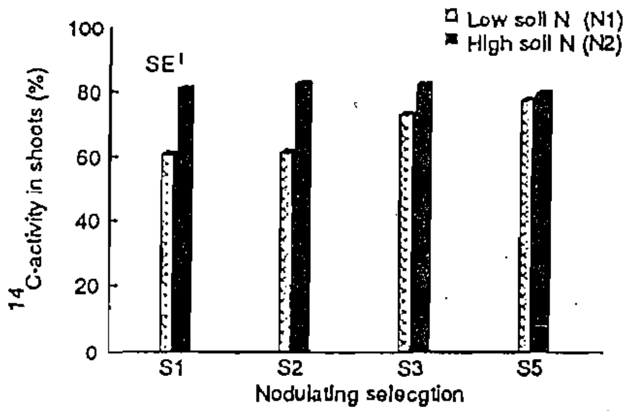

Figure 4. Proportion of ^{14}C-photosynthate retained in shoots of different nodulating selections of chickpea cv ICC 5003 grown at two soil N in pots

S1-S2 low nodulating selection
S3-S5 high nodulating selection

C_2H_4 g^{-1} root in S1 and 2540 n mol C_2H_4 g^{-1} root in S5). However, nitrogenase activity per unit nodule mass decreased with the increasing degree of nodulation selection (S1 = S3 $\geq$ S4 = S5). These results indicate that nitrogen fixation (C_2H_2 reduction) in chickpea was drastically reduced by available nitrogen status of the soil. Such changes in nitrogenase activity were not observed by Rupela and Johansen [6] because the lowest N level used in their experiments (28 μg N g^{-1} soil) was high compared with 14 μg in this experiment. To increase biological nitrogen fixation in chickpea optimization of soil mineral N levels are necessary. This could be achieved through the use of appropriate crop rotation including non-legume crops, incorporation of plant residues which can immobilize mineral N, and selection of host-plants that can fix N_2 in the presence of higher mineral N levels.

The proportion of ^{14}C-photosynthate activity retained in shoots increased with the degree of nodulation of plants grown on soil with low nitrogen status (Fig 4). In contrast, at the higher soil nitrogen level there was no difference in the amount of ^{14}C-photosynthate activity in the shoots.

TABLE I. NITROGENASE ACTIVITY AFTER HARVEST (μ MOL C_2H_4 PLANT^{-1} h^{-1}) IN LOW AND HIGH NODULATING SELECTIONS OF CHICKPEA CV. ICC 5003 GROWN IN POTS AT TWO SOIL N LEVELS

Selection	N1	N2	Mean
S_1 (low)	0.77	0.24	0.51
S_2 (low)	2.62	0.04	1.33
S_3 (high)	2.80	0.29	1.54
S_5 (high)	3.30	0.15	1.72
SE ±	0.560	0.396	
Mean	2.37	0.18	
SE ±	0.280		

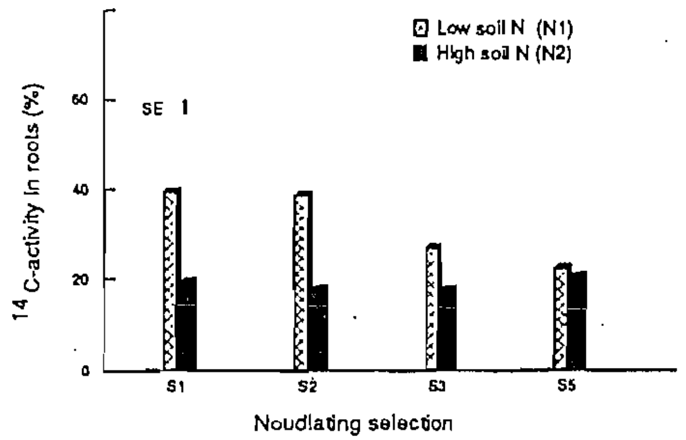

Figure 5. Proportion of ^{14}C-photosynthate in roots (roots and nodules) of different nodulating selections of chickpea cv ICC 5003 grown at two soil N levels in pots.

S1-S2 low-nodulating selection
S3-S5 high- nodulating selection

However,the mean specific ^{14}C-photosynthate activity (g^{-1} shoot) at N_1 level was significantly ($P < 0.05$) higher in S5 selection (36.3×10^4 dpm g^{-1})than in S1 (19.1×10^4 dpm g^{-1}). Similar results were not observed at N_2 level. The proportion of ^{14}C-photosynthate translocated to the roots decreased with increasing nodulation rank and also with increased soil mineral N level(Fig 5). All the chickpea selections grown at high soil nitrogen level except S_5, showed significantly ($P < 0.01$) lower translocation of ^{14}C-photosynthate activity in roots than plants grown at low nitrogen level.

Low nodulating selections of chickpea grown at low soil N levels were unable to fulfil their N requirements through biological nitrogen fixation as evidenced by their significantly increased biomass yield when grown at high soil N. Low and high nodulating selections produced similar root mass under low and high soil N conditions and low nodulating selections (S_1 and S_2) fixed less N than high nodulating selections (S_3 and S_5). Low nodulating selections had a higher root/shoot ratio than high nodulating selections suggesting that when low nodulating selections could not meet their N

TABLE II. SPECIFIC ^{14}C-PHOTOSYNTHATE ACTIVITY IN SHOOTS OF LOW AND HIGH NODULATING SELECTIONS OF CHICKPEA CV. ICC 5003 GROWN IN POTS AT TWO SOIL N LEVELS

Selection	Specific ^{14}C-photosynthate activity (dpm x 10^{-4} g^{-1} shoot)		
	N1	N2	Mean
S_1	19.1	51.6	35.4
S_2	24.7	46.6	35.7
S_3	36.0	37.4	36.7
S_5	36.3	46.1	41.2
SE±	2.80	1.98	
Mean	29.0	45.5	
SE±	1.40		

requirements through BNF at low soil N levels, these plants invested more carbon in increased root production so as to better exploit soil N.

Furthermore, the proportion of ^{14}C-photosynthate activity in shoots (including leaf) was significantly lower in S_1 than in S_5 selection, under low nitrogen level ($S_1 = S_2 \leq S_3 = S_5$). The increased demand for carbon in the roots of low nodulating selections in contrast to that in high nodulating selections resulted in a proportionately higher translocation of ^{14}C-photosynthate to the roots. Thus, the argument that BNF incurs a substantial drain of photosynthate is not applicable to chickpea. However, to obtain an accurate carbon-budgeting we also need to consider its loss through root respiration. To the contrary, it appears that poorly nodulated chickpea requires a greater investment of photosynthate in root development presumably to better enable acquisition of mineral N. The yield of low nodulating selections even with high fertilizer application in fields is not comparable with that of high nodulating chickpea selection with no fertilizer application [8]. Selection of host-plants with high biological nitrogen fixation efficiency coupled with management of soil N levels can sustain chickpea yields in the semi-arid tropics (SAT).

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