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Nodule Activity and Nitrogen Partitioning to Seeds of Ratooned and Non-Ratooned Winged Bean Plants Grown With and Without Support

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Abstract

A field experiment was conducted, and continued for three crop growth cycles, to investigate the effects of support systems and ratooning on the nodule activity and partitioning of nitrogen in relation to seed yield of winged bean grown under humid tropical conditions. Allantoin was the predominant export product of N_2 fixation among N-solutes in xylem exudates. Nitrate-N and amino-N were the intermediate and least export product of N_2 fixation. Plants grown on support height of 2 m and ratooned at 133 DAG accumulated significantly higher leaf N at vegetative growth stages and consequently N partitioning to pod was substantially increased. Non-ratooned plants grown on support height of 2 m obtained higher nitrogen fixation compared with those on support height of 1 m and control plants. Plants grown on 2 m support height and ratooned at 133 DAG recorded the highest cumulative seed yield (6.26 t ha^{-1}) over three crop cycles. The lowest cumulative seed yield (1.28 t ha^{-1}) was obtained from unsupported control plants ratooned at 175 DAG. The results revealed that winged bean plants grown on a 2 m support height as ratooned crop is technologically feasible and viable.

Keywords: Nodule, nitrogen, ratooned, seed, support, winged bean

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1. Introduction

Winged bean [*Psophocarpus tetragonolobus* (L) DC] is an indeterminate, climbing legume which requires some kind of support to achieve high yields. The major constraint to large scale production of winged bean is the need for trellising (Eagleton et al., 1985) which produces a higher production cost. After harvesting of young or mature pods the plants can be cut-off and the pollarded root stock will produce a ratoon crop. Ratooning is a means of spreading out the initial cost of construction of support. Ratooning would apparently cause a temporary set back in photosynthetic production, but the remaining photosynthetic area and the stored carbohydrates in the undisturbed root system and older shoots could sustain the plant.

Nitrogen fixation is second only to photosynthesis in terms of importance to the growth and development of plants (Gustavo, 1997). Plants grown on 1 and 2 m support heights produced higher seed yield relative to unsupported control plants. This high seed production was associated with higher net assimilation rate and dry matter production (Motior et al., 1997). There is experimental evidence available on seed production of non-ratooned winged bean as reported by Ridzwan and Hoe (1982) and Poi and Ghosh (1986), but little is known about the effects of ratooning on nitrogen partitioning and seed production. Therefore, our experiment was designed to study the responses of winged bean to ratooned practices and also to investigate its effects on nodule activity and partitioning of nitrogen in relation to seed yield in winged bean grown on support systems of varying heights.

2. Materials and Methods

The experiment was conducted during January 1996 to January 1997 at Field 2, University Putra Malaysia, Serdang (latitude 30° 2' N and longitude 101° 42' E), Malaysia. The 3 × 4 factorial experiment consisted of three levels of support systems and four levels of ratooning schedules. The support systems consisted of: i) unsupported control, ii) 1 m height wire trellis and iii) 2 m height wire trellis. Ratooning schedules were as follows: i) without ratooning (control), ii) ratooning done at 133 DAG, iii) ratooning done at 154 DAG and iv) ratooning done at 175 DAG. The treatment combinations were laid out in a split plot design with four replications. Support systems and ratooning schedules were placed in the main and sub-plots, respectively.

Seed sowing was completed in one day. After harvesting of mature pod from the main crop, ratooning schedules were implemented according to ratooning regime. The plants were cut at a height of about 30 cm above the ground surface (i.e. 8–10 nodes remained in old plant stock) for all ratooned treatments. After completion of one ratoon cycle the main plant was cut again for the next ratoon cycle (i.e. 2nd ratoon). Plants were cut at 10–12 nodes above from old stock. All cultural practices followed those described earlier by Motior et al. (1998).

At 15 day intervals, beginning from 42 days (or day 56) to day 126, N_2 fixation was estimated by the acetylene reduction assay (ARA) method. The accuracy of ARA, which is a closed system of Oti-Boateng and Silsbury (1993), has received serious criticisms (Minchin et al., 1994), since acetylene itself can induce a decline in nitrogenase activity during a 1-h incubation period of the assay (Minchin et al., 1983). In this experiment extreme care was taken to dig out the entire root system from the ground with minimum loss of nodules. The volume of excavation of the root systems was about 0.4 m^3 and more than 95% nodule was collected. Details of the ARA were as described by Motior et al. (1998). After ARA gas sample collection, the nodules were dried in an oven at 75°C for 48 h weighed for total nodule dry weight calculation.

The xylem exudate technique is another method of estimating nodule activity, and in the present study this technique is to confirm the ARA results. The concentrations of allantoin (ureide-N), amino-N and nitrate-N in the exudates were determined colorimetrically on UV-VIS spectrometer (model-1201) using the procedures described by Young and Conway (1942) and Cataldo et al. (1975), respectively, as modified by Peoples et al. (1989). Xylem exudate (sap) was collected from eight plants in each treatment at 15 day intervals from 42 days until 126 days for main crop cycle and 112 days for first ratooned cycle.

The relative distribution of nitrogen among plant organs was determined during vegetative (pre-flowering) and reproductive stages (post-anthesis) for observing N partitioning patterns. After determination of dry matter plant samples were ground and analyzed for total nitrogen. Details of the micro-Kjeldhal and the N determination using an auto-analyzer were as described by Bremner and Mulvaney (1982) and Motior et al. (1998). The N accumulation in various plant tissues was calculated using the following formula: N accumulation = N concentration × dry matter yield.

Seed yields were also recorded. The data were subjected to statistical analyses, appropriate for the experimental design using the Statistical Analysis System (SAS, 1987).

3. Results

Nodule activity of ratooned winged bean grown on different support systems

Nodule dry weight was affected significantly by support systems, ratooning and their interaction. Plants grown on a support height of 2 m produced the

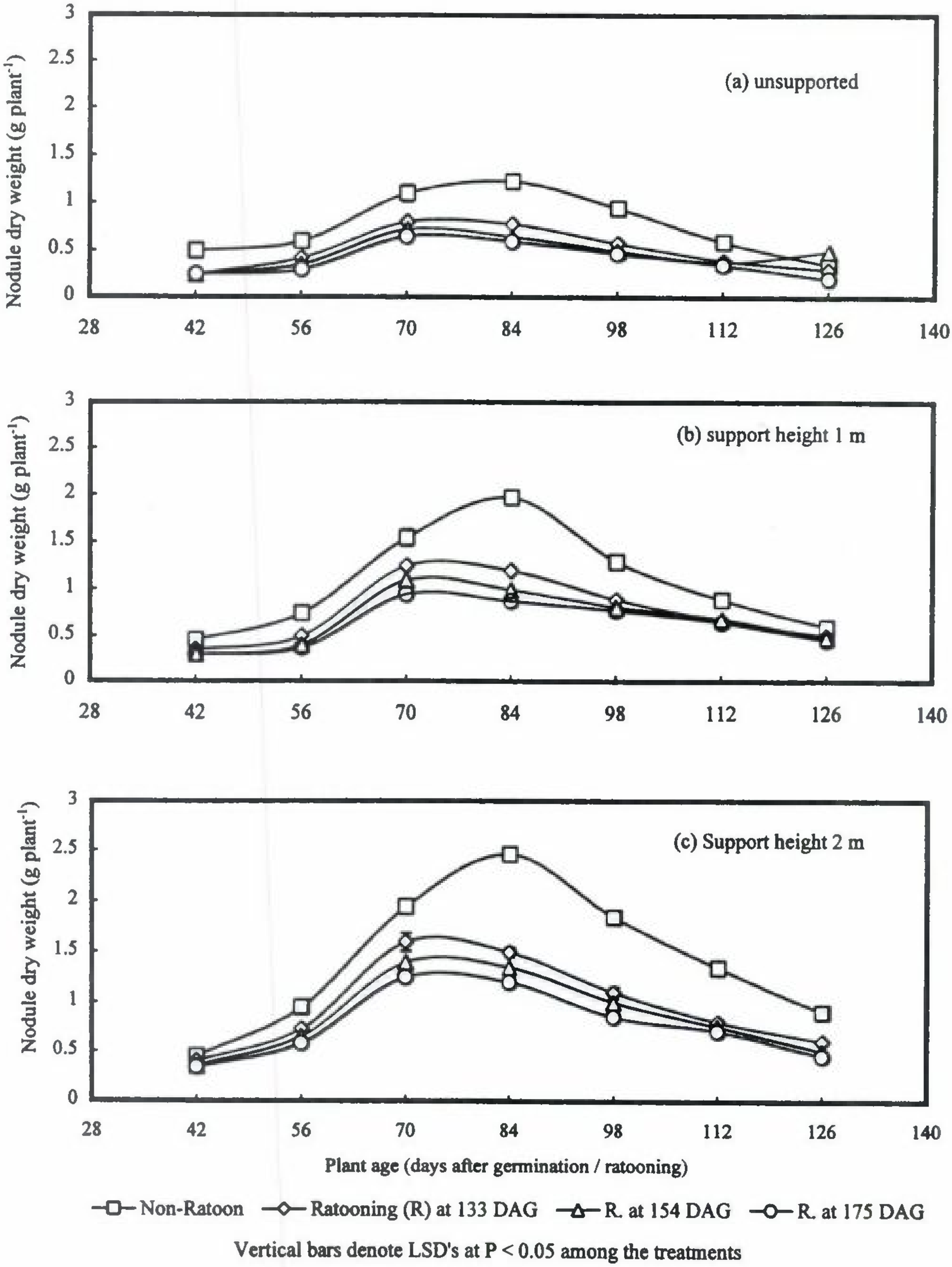

Figure 1. Nodule dry weight of ratooned winged bean grown on support systems.

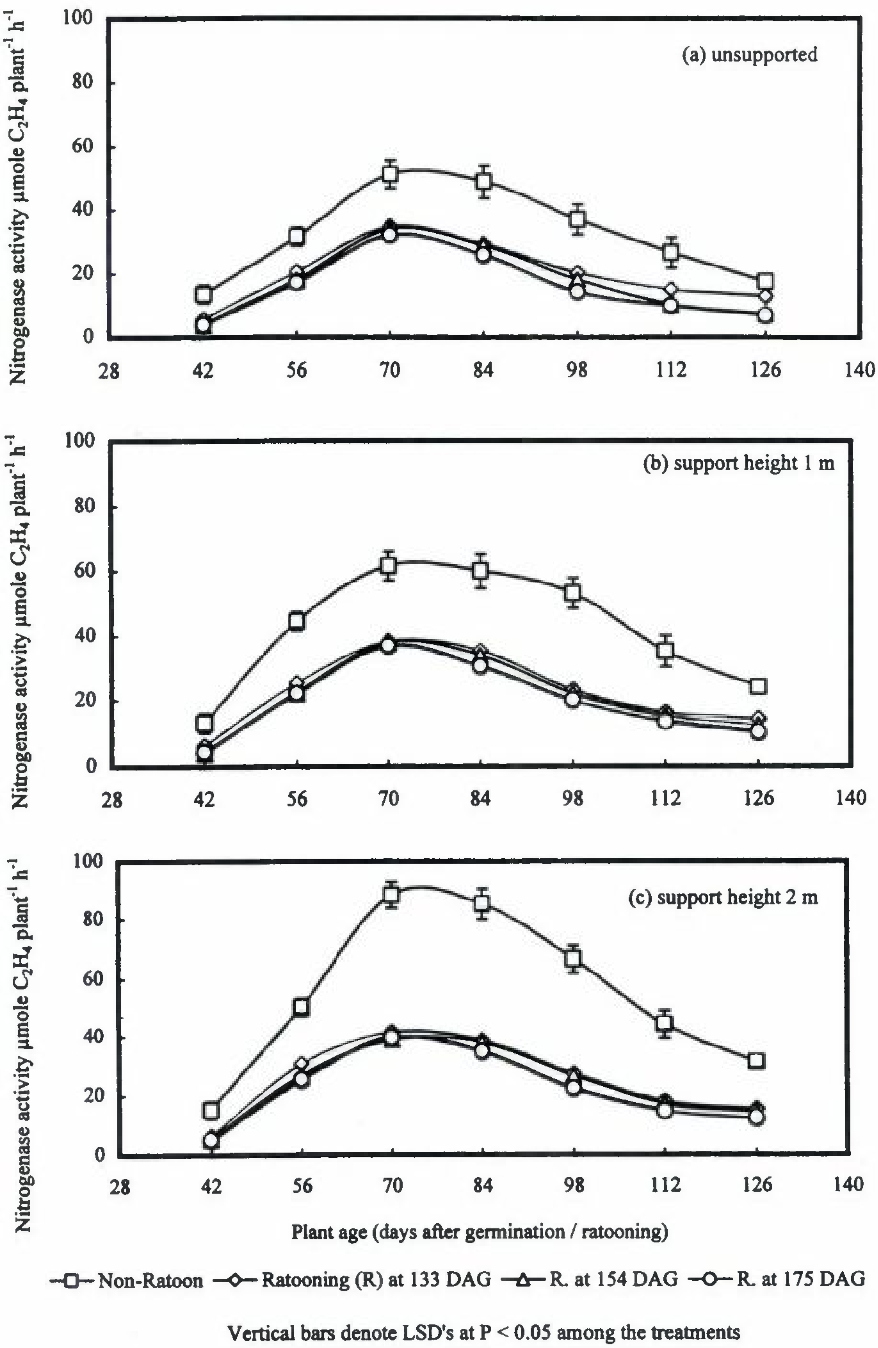

Figure 2. Nitrogenase activity of ratooned winged bean grown on support systems.

largest nodule mass which was significantly higher than those with support at 1 m and unsupported plants (Fig. 1). Unsupported plants recorded the least nodule weight per plant while those with 1 m support produced intermediate response. Under non-support systems, non-ratooned plants produced the highest nodule dry weight (Fig. 1a). Nodule dry weight of this treatment was significantly higher than those of plants ratooned at 133, 154 and 175 DAG. No differences were observed on nodule mass for plants ratooned at 133, 154 and 175 DAG. The above results were similarly shown by plants grown on support height of 1 and 2 m (Fig. 1b and c). Plants grown on support system produced maximum nodulation at early pod formation stage (84 days) while unsupported plant produced maximum nodulation at flowering time (70 to 84 days) and began to decline at full flower or at the beginning of pod filling (Fig. 1).

Nitrogenase activity was closely associated with nodulation and differed markedly by support systems, ratooning and their interaction. Plants grown on 2 m support height obtained superior nitrogenase activity which was significantly higher than those grown on 1 m support height which in turn was higher than unsupported plants (Fig. 2). Unsupported non-ratooned plants had higher nitrogenase activity than ratooned crops (Fig. 2a). Ratooning at 133 DAG recorded similar nitrogenase activity as ratooning at 154 and 175 DAG. The above results were similarly shown by the plants grown on 1 and 2 m support heights (Fig. 2b and c). Nitrogenase activity increased at the onset of flowering or at initial pod filling and declined onwards in plants grown on support systems. On the contrary unsupported non-ratooned plant recorded a peak nitrogenase activities 14 days before flowering and at early pod formation in unsupported ratooned plants. Evidently, nitrogenase activity almost followed the same trend as nodulation. This result indicates that nitrogenase activity is directly dependent on nodule mass (Fig. 2).

N solutes concentration in xylem exudates of ratooned winged bean grown on different support systems

Support systems, ratooning and their interaction had significant influence on allantoin (ureide-N), nitrate-N and amino-N concentrations. Plants grown at a support height of 2 m produced significantly higher concentrations of ureide-N compared to those on 1 m support and unsupported plants (Fig. 3). Plants supported at 1 m in height recorded a significantly higher amino-N (Fig. 4) and nitrate-N (Fig. 5) while those on support height of 2 m and unsupported plants recorded lower amino-N and nitrate-N. Support height had no consistent effects on amino-N and nitrate-N. Under non-support system, allantoin was unaffected by ratooning (Fig. 3a). For plants grown on support height of 1 m, non-ratooned plant produced significantly higher allantoin than those of

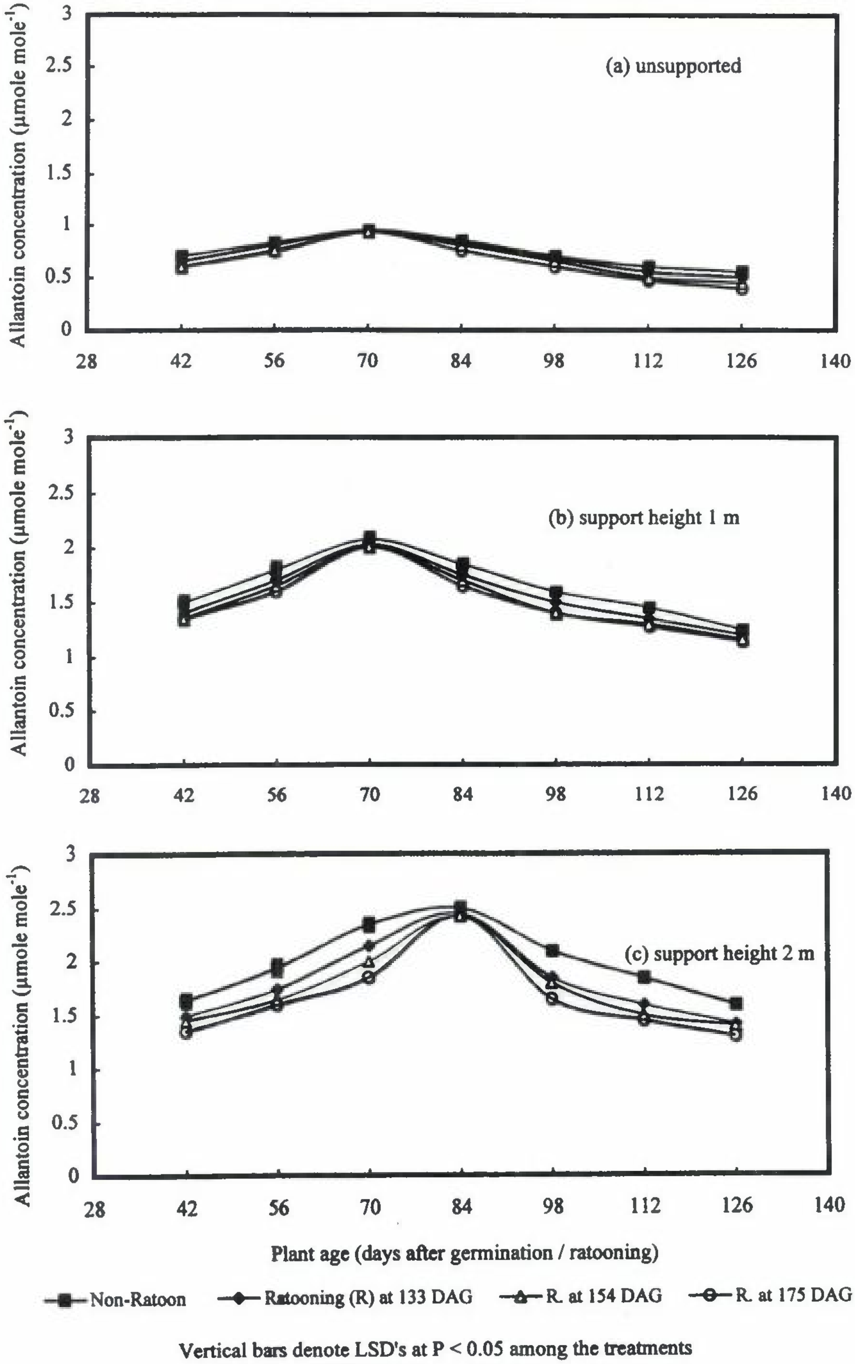

Figure 3. Allantoin concentration in xylem exudates of ratooned winged bean grown on support systems.

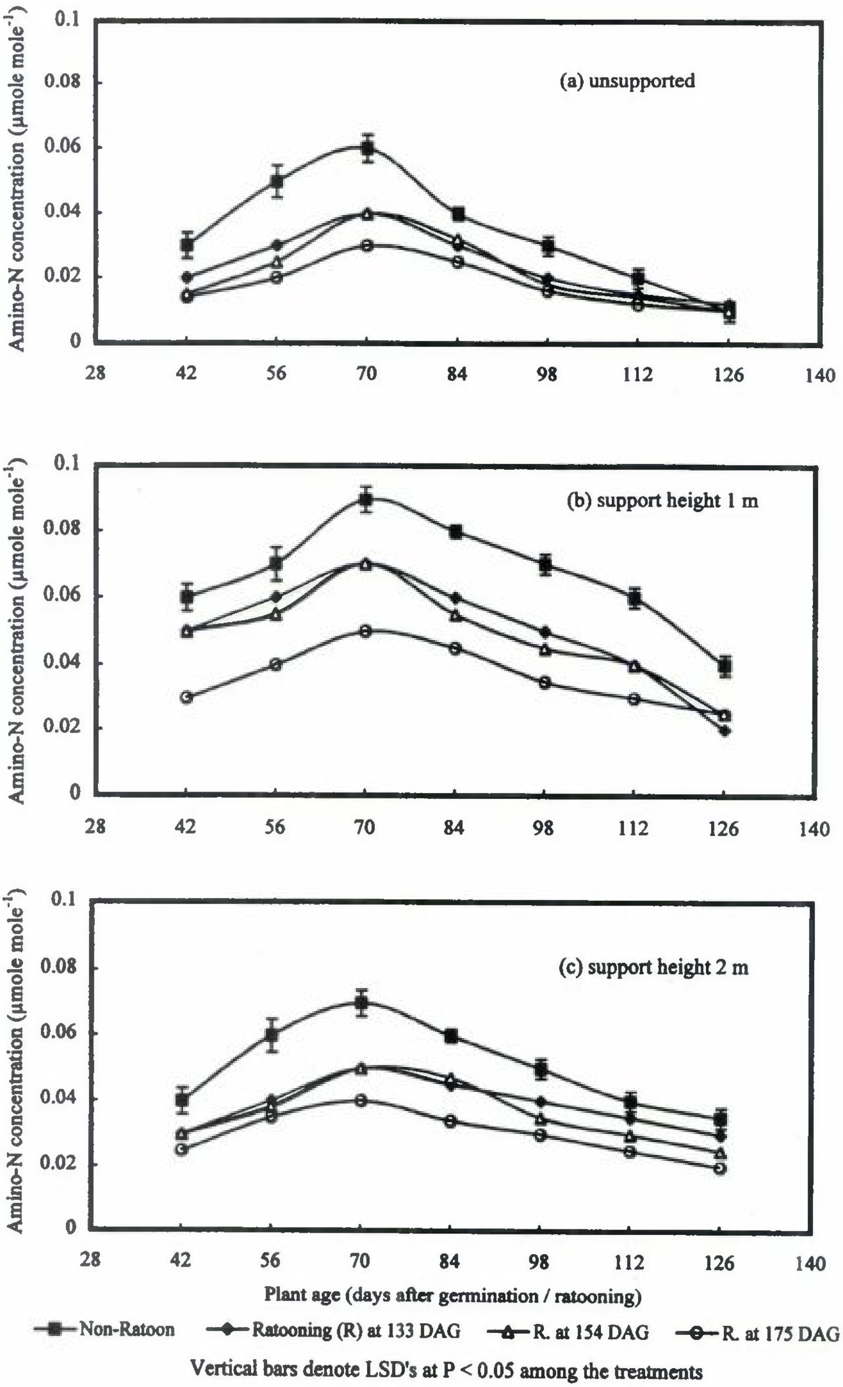

Figure 4. Amino-N concentration in xylem exudates of ratooned winged bean grown on support systems.

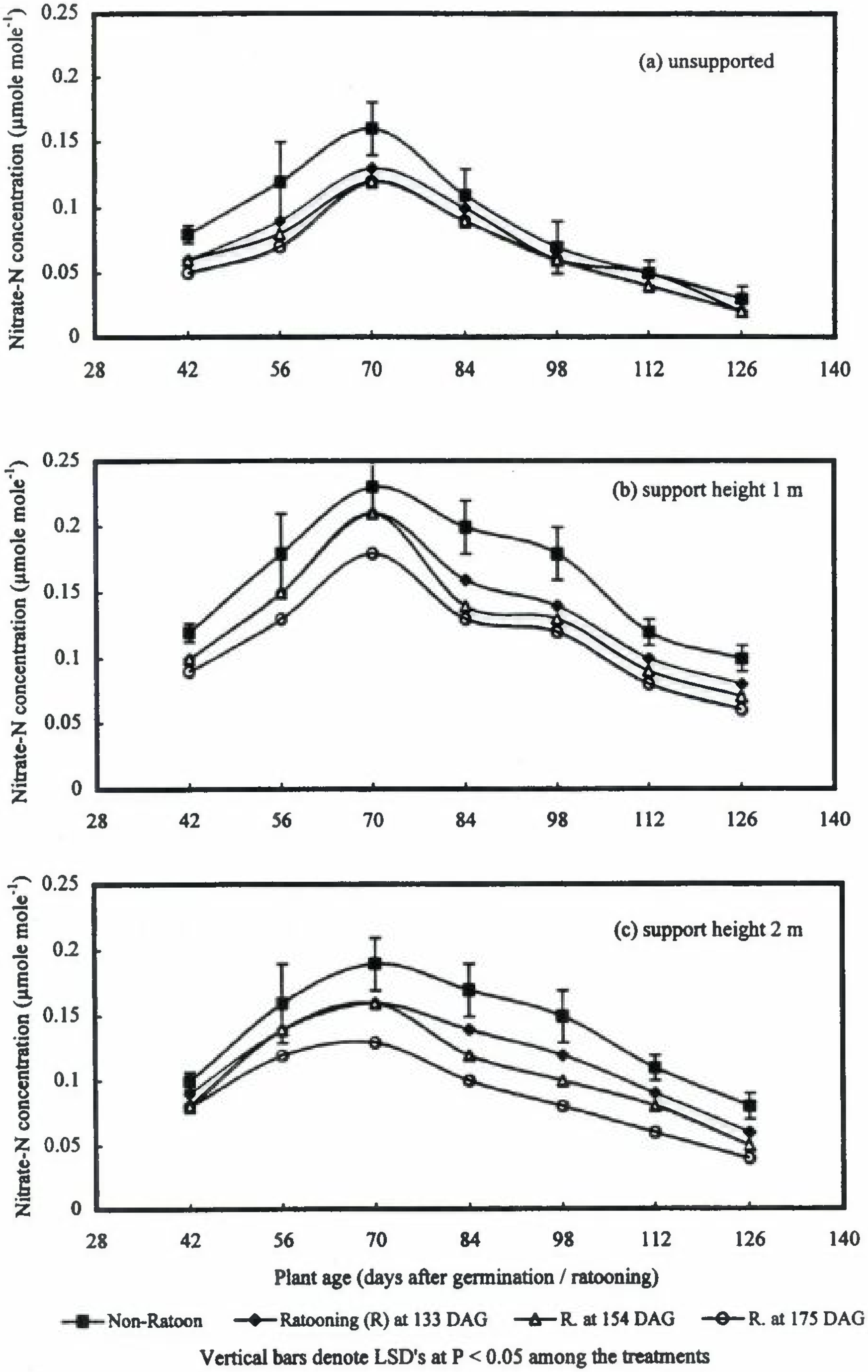

Figure 5. Nitrate-N concentration in xylem exudates of ratooned winged bean grown on support systems.

ratooned plants (Fig. 3b). Ratooning at 133, 154 and 175 DAG produced similar effects on allantoin concentration. The above results were similarly shown by plants grown on support height of 2 m (Fig. 3c). Unsupported non-ratooned plants contained significantly higher nitrate-N and amino-N, respectively, compared with ratooned crops (Fig. 4a and 5a). Similar results were shown by plants grown on support height of 2 m and 1 m (Fig. 4b, c and 5b, c). Irrespective of ratooning, allantoin concentration reached a peak at initial pod formation stage (day 84) for plants grown on a support height of 2 m and thereafter declined sharply until the end of crop growth. In plants grown on support height of 1 m and unsupported plants, concentration of allantoin reached a maximum at flowering stage and thereafter declined gradually (Fig. 3a). Nitrate-N and amino-N concentrations of xylem exudates reached a peak at day 70 for all types of plant and thereafter the N solutes declined sharply only in supported plants.

Nitrogen partitioning of ratooned winged bean grown on different support systems

Leaf, stem and pod N was affected by support systems, ratooning and their interaction. Unsupported plants accumulated significantly higher stem N at both vegetative and reproductive stages (Fig. 6) compared to supported plants. Reproductive leaf N was also higher for unsupported plants compared to supported plants of 1 and 2 m. Plants grown on support height of 2 m produced the highest vegetative leaf N and pod N which was significantly higher than that of support height of 1 m and unsupported plants. Plants grown on support height of 1 m recorded moderate N in all components.

Under non-support systems, stem N did not show any significant response among ratooned and non-ratooned plants at both vegetative and reproductive stages (Fig. 6a). At vegetative stage, ratooning at 133 DAG produced similar leaf N as ratooning at 154 and 175 DAG and these were significantly higher than non-ratooned plants. At reproductive stage, non-ratooned plant produced higher leaf N which was significantly different from ratooning at 133, 154 and 175 DAG. Similar pod N contribution was obtained from ratooning at 133, 154 and 175 DAG and these were significantly higher compared to non-ratooned plant. For plants grown on support height of 1 m, ratooning at 175 DAG accumulated higher stem N at vegetative stage whilst at reproductive stages non-ratooned and ratooning at 175 DAG produced similar stem N which were significantly higher compared to ratooning at 133 and 154 DAG (Fig. 6b). Ratooning at 133 DAG produced higher leaf N at vegetative stage whilst non-ratooned recorded similar leaf N as ratooning at 175 DAG at reproductive stage which were significantly different from ratooning at 133 and 154 DAG.

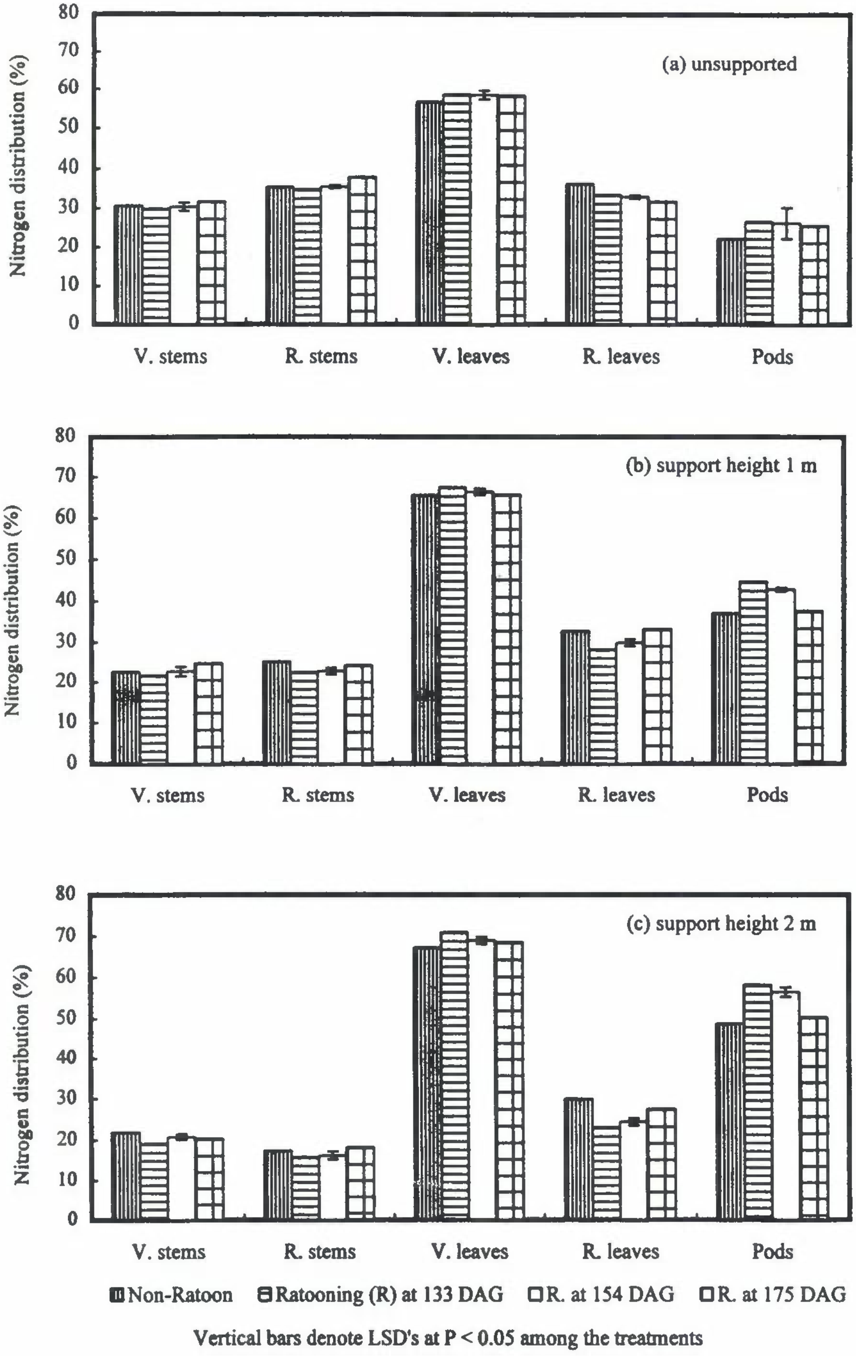

Figure 6. Relative distribution of nitrogen among ratooned winged bean plant organs grown on support systems.

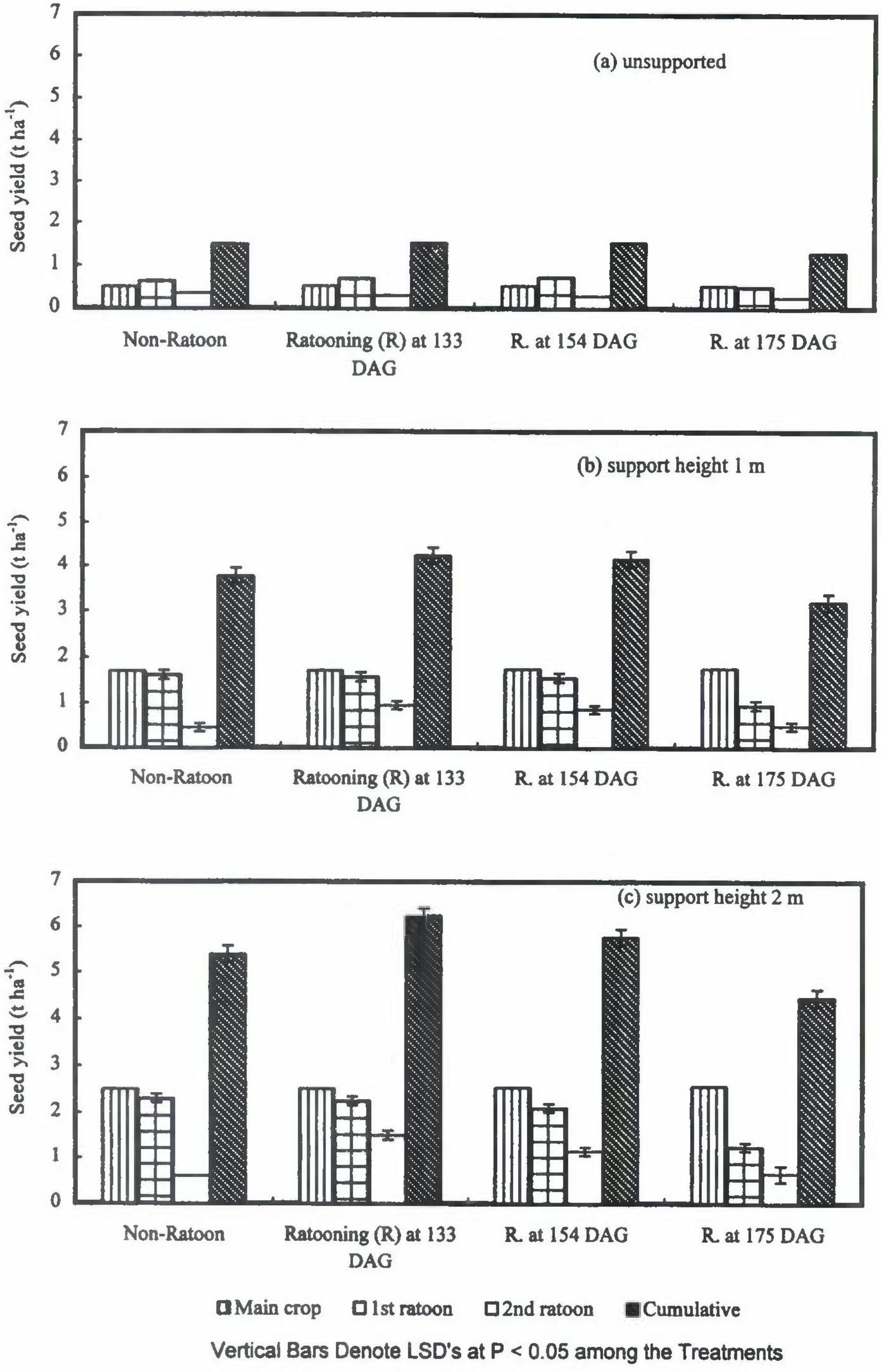

Figure 7. Seed yield of ratooned winged bean grown on support systems.

Ratooning at 133 DAG accumulated the highest pod N which was significantly superior compared to non-ratooned and ratooning at 154 and 175 DAG. Non-ratooned plants recorded the lowest pod N and at par to ratooning at 175 DAG. The above results were similarly shown by the plants grown on support height of 2 m (Fig. 6c).

During vegetative stages leaves were the major site of N accumulation. N partitioned to leaves comprised of 57 to 71% of total plant N. Nitrogen contents in leaf declined by 21–48% during the reproductive phase. Out of total N accumulated per plant the pod gained 22–58%. During the reproductive phase, there was a greater decline in leaf N (48%) and least decline in stem N (4%) for plants ratooning at 133 DAG under support systems of 2 m in height (Fig. 6c). The decline was the contribution of N for pod development. However, contribution of leaf N at vegetative and pod N at reproductive phases were higher in ratooned plants compared with non-ratooned crop. Stem N contribution towards pod was not observed at all in case of unsupported plants irrespective of ratooning (Fig. 6).

Seed yield and yield components of ratooned winged bean grown on different support systems

Three crop cycles of winged were grown and the cumulative seed yield was the combination of main crop yield (i.e. before execution of ratooning schedule) plus the first and second ratooned cycles. Support systems, ratooning and their interaction had a significant effect on seed yield. Plants grown on support height of 2 m recorded the highest cumulative seed yield of 6.26 t ha^{-1} (Fig. 7). Seed yield of plants subjected to this treatment was significantly higher than that of support height of 1 m which in turn produced higher seed yield than those of unsupported plants.

Unsupported non-ratooned plants produced similar seed yield as ratooning at 133 and 154 DAG from first and second cycle and as well as cumulative seed yield (Fig. 7a). Seed yields of plants given these treatments were significantly higher than those of ratooning at 175 DAG. For plants grown on support height of 1 m, ratooning at 133 DAG produced similar seed yield as non-ratooned and ratooning at 154 DAG (Fig. 7b), from the first ratooned cycle. In the second ratooned cycle, ratooning at 133 DAG produced higher seed yield which was significantly different than those of non-ratooned and ratooned at 154 and 175 DAG. Ratooning at 133 DAG produced similar cumulative seed yield as ratooning at 154 DAG which were significantly higher than those of other treatments (Fig. 7b). The above results were similarly shown by plants grown on support height of 2 m (Fig. 7c).

Pods per plant, seeds per pod, 100 seed weight and pod length was significantly affected by support systems. Pods per plant was also affected by ratooning and their interaction effect. Seeds per pod, 100 seed weight and pod length was unaffected by ratooning and their interaction. The largest number of pods per plant was produced by plants grown on support height of 2 m and unsupported control plant recorded the least number of pods whilst support height of 1 m produced intermediate pod number per plant (Table 1). Under non support systems, ratooning at 133 DAG produced similar number of pods as ratooning at 154 DAG. The number of pods per plant subjected to these treatments were significantly higher than those of non-ratooned and ratooned at 175 DAG. The above results were similarly shown by plants grown on support height of 1 and 2 m.

Table 1. Seed yield attributes of ratooned winged bean grown on different support systems

Support systems × ratooning (R)	Pods plant^{-1}	Seeds pod^{-1}	100 Seed weight (g)	Pod length (cm)
Unsupported				
Non-ratooned	5.00 b	10.00 a	30.00 a	19.25 a
R. at 133 DAG	6.00 a	10.00 a	30.00 a	19.05 a
R. at 154 DAG	6.00 a	10.00 a	30.00 a	19.30 a
R. at 175 DAG	5.00 b	10.00 a	30.00 a	19.30 a
Mean	5.50 C	10.00 B	30.00 B	19.23 C
Support height 1 m				
Non-ratooned	10.00 c	11.00 a	37.00 a	24.00 a
R. at 133 DAG	13.00 a	11.00 a	37.10 a	24.50 a
R. at 154 DAG	12.00 b	10.25 a	37.13 a	24.00 a
R. at 175 DAG	10.00 c	11.00 a	37.00 a	24.00 a
Mean	11.25 B	10.81 B	37.06 A	24.13 B
Support height 2 m				
Non-ratooned	15.00 c	12.00 a	37.20 a	25.50 a
R. at 133 DAG	18.00 a	12.13 a	37.25 a	25.00 a
R. at 154 DAG	17.00 b	12.13 a	37.23 a	25.00 a
R. at 175 DAG	15.00 c	12.00 a	37.00 a	24.00 b
Mean	16.25 A	12.07 A	37.17 A	24.88 A

Values in the same column followed by the same small letters are not significantly different with in the same support system at the 0.05 level by DMRT. Means of yield attributes for each support height within a row followed by the same capital letters are not significantly different at the 0.05 level by DMRT.

4. Discussion

Irrespective of support systems, non-ratooned plants produced similar seed yield as ratooned from main crop and the second crop cycle, but this yield was drastically reduced in the third cycle. Plants grown on support height of 2 m with ratooning at 133 DAG produced high seed yield from the main crop, and the first ratooned crop. The yield was even higher from the second ratooned cycle. The higher seed yield produced by 2 m support plants ratooned at 133 DAG was largely associated with increased number of pods per plant, pod length and seeds per pod (Table 1). Non-ratooned plants were not able to produce more pods due to lower leaf N partitioning (Fig. 6) lower N partitioned to pods (Fig. 6), earlier leaf senescence (Motior et al., 1998) and resulting in source limitation. In fact, the highest cumulative seed yield was achieved by the contribution of second ratooned crop cycle (Fig. 7).

At the vegetative stage 57–71% of total plant N was partitioned to the leaf, which confirms the results of Muchow et al. (1993), and the assumption in the soybean model of Sinclair (1986). Concentrations of N in above-ground plant parts decreased consistently from the onset of flowering, with a rapid decrease from the start of pod filling until maturity. Leaf N concentration decreased more than stem N concentration. The decline in leaf N concentration presumably reflected the mobilization of N to the developing pods. Peoples et al. (1983) found similar results in several grain legumes. Bushby and Lawn (1992) also reported similar results for mungbean genotypes, and showed that leaf N concentration decreased consistently from the start of pod fill until maturity.

The proportion of grain N derived from mobilization varied between 22% and 58%. The mobilization was related to the amount of N available for redistribution rather than the ability of plants to continue N_2 fixation during pod filling. In the present study, the decline in leaf N in supported plants was associated with grain development and also with the senescence of leaves due to aging. The main cause of the decline in leaf N in unsupported and non-ratooned plants was the early senescence of leaves due to mutual shading.

Nodulation and N_2 fixation rates of winged bean were enhanced by provision of 1 or 2 m support height compared with unsupported plants averaged over the ratooning. Similar results were observed by Motior et al. (1998) in non-ratooned winged bean under different support height. Nodule development was more active with the onset of flowering or at initial pod formation and thereafter declined for support systems in main crop. Ratooned plants failed to produce more nodule and consequently nitrogenase activity was relatively low. Motior et al. (1998) reported that N_2 fixation rate was directly related to nodulation and net photosynthetic efficiency. In ratooned crops, the root system was older and nodule formation was poor compared to the main crop, irrespective of support systems. Therefore, nitrogenase activity of ratooned plants could not achieve it full potential compared to main crop. Bushby and Lawn (1992) found that in one mungbean genotype which behaved as perennial produced that there was less nodules and were very much reduced in nitrogenase activity during the second cycle.

In this study xylem exudate technique was used as an alternative method of estimating nodule activity and this technique confirmed the ARA results (Wan Mohamad et al., 1993). Allantoin (ureide N) was the predominant export product recovered in xylem exudates, with the remaining N solutes comprising nitrate-N and amino-N. This result is consistent with the findings of Wan Mohamad et al. (1993). Peoples and Herridge (1990) reported that winged bean transported ureides as a major nitrogenous component of xylem sap. The accumulation trend of allantoin followed exactly similar patterns of nitrogenase activity of ratooned and non-ratooned winged bean irrespective of support systems (Fig. 2).

Based on the above findings it is evident that the indeterminate climbing winged bean grown on a 2 m support height accumulated substantial amounts of total N per plant compared with those on support height 1 m and unsupported plants. It is concluded that total N accumulation, and leaf N played an important role in vegetative growth and seed yield of winged bean. Seed production of winged bean can be increased by more than 5-fold by adopting a 2 m support height and appropriate ratooning practices. The optimum time of ratooning at 133 days after germination under support system of 2 m height maximized seed yield per unit area per year, thereby spreading out the cost of trellising over three crop cycles.

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