Indian J. Plant Physiol., Vol. 8, No. 4, (N.S.) pp. 354-358 (Oct.-Dec., 2003)

STUDIES ON NITROGEN FIXATION CHARACTERS IN SOME VARIETIES OF COWPEA

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SUMMARY

Under a polythene bag culture experiment, three high-yielding varieties of cowpea (*Vigna unguiculata* L.) of Karnataka, namely KBC-2, V-322 and GC-2 were evaluated for various nitrogen fixation-related characters at 35, 50 and 65 DAS (days after sowing) during kharif. The acetylene reduction activity (ARA) of root nodule nitrogenase and leghemoglobin (Lb) contents were higher at 35 DAS, but they decreased linearly with an increase in growth stages in all the varieties. The highest mean ARA was noticed in KBC-2 (11.6 µ moles C_2H_4 formed/h/g fw of nodules) and the lowest was in V-322 (7.1 µ moles C_2H_4). The mean Lb content was found highest in GC-2 (1.26 mg/g fw of nodules) and lowest in V-322 (1.18 mg). The shoot and root ureide content and the relative abundance of ureides (RAU) were also higher at 35 DAS in all the varieties. The nitrate-N content increased while the total-N content in the shoots decreased with the increased growth stages. The highest nitrate-N content was noticed in KBC-1 (306 µg/g dry w) while the lowest was in V-322 (204 µg/g dry w). The highest mean total-N content was observed in KBC-2 (3.12%) whereas the lowest was in GC-2 (2.50%). The overall results suggested that KBC-2 was superior in nitrogen fixation among the cowpea varieties studied.

Key words: **Cowpea, leghemoglobin, nitrogenase, nitrogen fixation, ureides.**

INTRODUCTION

N_2 fixation occurs in root nodules containing bacteria of the genus *Rhizobium*. These bacteria are responsible for synthesizing the enzyme, nitrogenase. The plant donates substrates for the production of electrons and ATP which are the requirements for N_2 fixation. N_2 fixation is estimated in a variety of ways that include determination of Kjeldahl-N content of plants, root nodule leghemoglobin content, (Johnson and Hame 1973) acetylene reduction activity (ARA) of root nodule nitrogenase and crop yield (Hardy *et al.* 1968).

A number of tropical legumes synthesize ureides (allantoin and allantoic acid) from currently fixed N_2 in nodules and use these compounds for transport and storage of N in N_2 fixing soybeans suggesting a special association between N_2 fixation and ureide formation (McClure and Israel 1980). The relative abundance of ureides (RAU) has been found to be closely related to the N derived from symbiotic N_2 fixation in wide range of genotypes (Avline *et al.* 1995). This paper reports the evaluation of some of the promising cowpea (*Vigna unguiculata* L.) varieties of Karnataka for nitrogen fixation related characters, distribution pattern and changes in the levels of ureides, different forms of nitrogen in plant parts which could be exploited further in breeding programmes.

MATERIALS AND METHODS

Plant material and growth: Seeds of three high-yielding varieties of cowpea (*Vigna unguiculata* L.) namely KBC-2, V-322 and GC-2 were obtained from the AICRP

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(Pulses) of this University. The seeds were sown in 5 kg capacity perforated polythene bags containing field soil and the plants were grown under ambient conditions as per the standard agricultural practices during kharif. The plants intact with root nodules were harvested at 35, 50 and 65 days after sowing (DAS) and separated into shoot (leaf + stem) and nodulated root for analysis. Four replications were maintained for each variety and for each DAS.

Nitrogenase assay of nodulated roots: Nitrogenase activity in fresh nodulated roots was assayed by C_2H_2 reduction method (Hardy *et al.* 1968) using AIMIL-NUCON (Model-5700) gas chromatograph.

Estimation of leghemoglobion (Lb) content: After nitrogenase assay, the fresh nodules were detached from roots, extracted with 0.2 M Tris-acetate buffer, pH 4.0 centrifuged at 3000 rpm for 10 min and the supernatant was used for Lb estimation colorimetrically at 540 nm by the methods of Procter (1963).

Estimation of ureide content: The plant shoots and roots were dried at 60°C in an hot-air oven to a constant weight, extracted separately with 0.05M phosphate buffer, pH 7.5 and centrifuged at 10,000 rpm for 5 min at room temperature. The supernatants were used for ureide estimation as per the method described by Matsumoto *et al.* (1977).

Determination of relative abundance of ureides (RAU): The RAU was calculated as per the method of Herridge (1982) using the following relationship.

$$RAU = (Ureide\text{-}N/Ureide\text{-}N+Nitrate\text{-}N) \times 100$$

Estimation of nitrate-N and total-N content: The nitrate-N content in the shoot dried at 60°C in an hot-air oven to a constant weight was estimated by the phenol disulphonic acid method as described by Black (1965). The total-N content in the dried shoot was estimated by the micro-Kjeldahl method (Jackson 1973).

RESULTS AND DISCUSSION

Nodule nitrogenase activity: The ARA of nodule nitrogenase among the different cowpea varieties is presented in Table 1. The ARA was higher at 35 DAS, but it decreased linearly with an increase in growth stages in all the varieties. The highest mean ARA was noticed in KBC-2 (11.6 µmoles C_2H_4 formed/h/g fr. wt. of nodules), whereas the lowest was in V-322 (7.1 µmoles C_2H_4). The ARA was higher at initial stages (35, 50 DAS) but it decreased remarkably at later stage of growth (65 DAS) in all the varieties.

Table 1. Acetylene reduction activity of nodule nitrogenase in cowpea varieties at differnt stages of growth.

Variety (V)	Acetylene reduction activity[a] (µmol C_2H_4 formed/h/g fw nodules)			
	Days after sowing (DAS)			
	35	50	65	Mean (V)
KBC-2	16.45	13.70	4.62	11.6
V-322	15.20	5.60	0.59	7.10
GC-2	12.60	10.12	0.40	7.70
Mean (DAS)	14.8	9.8	1.8	
CD value at 5%	V = 1.0, DAS = 1.2; V × DAS = NS			

a: average of four replications

Among the various biological agents which fix atmospheric dinitrogen, the legume-*Rhizobium* symbiosis is the efficient system as it contributes most nitrogen to the ecosystem and food production. Nitrogenase, which reduces atmospheric dinitrogen to ammonia can be conveniently assayed by acetylene reduction method (Hardy *et al.* 1968). The acetylene reduction activity (ARA) of root nodule nitrogenase has been used by many workers earlier for measuring the nitrogen fixation rate in crop plants.

The decrease in ARA with an increase in growth stages may be attributed to the decrease in the leghemoglobin (Lb) content (Bhupinder *et al.* 1994) and increased nitrate-N content (Karunakaran *et al.* 1996). Similar decrease in ARA with an increase in growth stages was also reported earlier by several workers in soybean (Wych and Rains 1979) bean (Westermann and Kolar 1978) and chickpea (Koundal and Sinha 1992).

Nodule Lb content: The nodule leghemoglobin content also decreased with an increase in DAS and was found higher at initial stages of growth (35, 50 DAS) (Table 2). GC-2 showed the highest mean Lb content (1.26 mg/g fr. wt. of nodule) and the lowest was in V-322 (1.18 mg/g).

Table 2. Nodule leghemoglobin conten in cowpea varieties at different stages of growth.

Variety (V)	Leghemoglobin content[a] (mg/g fw nodule)			
	Days after sowing (DAS)			
	35	50	65	Mean (V)
KBC-2	1.45	1.46	0.86	1.25
V-322	1.44	1.04	1.06	1.18
GC-2	1.68	1.11	1.01	1.26
Mean (DAS)	1.52	1.20	0.97	
CD value at 5%	V = NS,	DAS = 0.15,	V × DAS = 0.29	

a: average of four replications

Similar decrease in Lb content with an increase in growth stages was also reported in *Phaseolus* sp. (Jamro *et al.* 1994), black gram (Singh *et al.* 1983) and chickpea (Bishnoi and Krishnamurthy 1991). The decreased Lb content may be partially attributed to the increased concentration of nitrate-N with an increase in the growth stages as the nitrate-N may bind with active Lb and make it inactive by forming a nitrosyl-Lb (Wych and Rains, 1979).

Ureide content: In general, the ureide content in shoot was higher than in root and it decreased with an increase in DAS (Table 3). The highest mean shoot ureide content was observed in V-322 (679 µg/g dry w) and the lowest was GC-2 (568 µg/g). As for stages, the highest mean shoot ureide content was recorded at 35 DAS (735 µg/g). The highest mean root ureide content was found in KBC-2 (513 µg/g) and the lowest was in GC-2 (399 µg/g). Amongst the stages, the highest root ureide content was at 35 DAS (485 µg/g).

Ureides are one of the important transport forms of nitrogen in some legumes (Herridge, 1982). The ureides (allantoin and allantoic acid) can make up 80 per cent of the total xylem sap nitrogen in nodulated plants which are entirely dependent upon symbiotic nitrogen fixation for growth. Plants which are less dependent upon symbiotic nitrogen fixation contain proportionately lower concentrations of ureides (Herridge, 1984). The mean ureide levels in shoots decreased with an increase in the growth stages in all the cowpea varieties, however, the root ureide levels were found to increase at later stages. The decrease in the ureide content with an increase in growth stages may be due to decrease in the nodule nitrogenase activity and Lb content as these are interrelated. Dakora and Felix (1995) reported a positive correlation between Lb and ureide content of xylem sap of cowpea and soybean plants. Atkins *et al.* (1984) in cowpea reported that an increase in the Lb content was coupled with increased enzymatic activities of ureide biosyntehsis.

RAU: Table 4 presents the RAU in shoots and roots of cowpea varieties at different DAS. In both shoots and roots, RAU decreased linearly with an increase in growth stages. The highest mean RAU was noticed in V-322 (77.9%) and lowest in GC-2 (64.8%). Similarly, the highest mean root RAU was found in GC-2 (73.3%) and the lowest in V-322 (69.1%). Both shoot and root showed the highest RAU at 35 DAS.

Table 3. Ureide content in shoot and root of cowpea varieties at different stages of growth.

Parameter/Variety	Ureides content in shoots[a] (µg/g dry w)				Ureides content in roots[a] (µg/g dry w)			
	Days after sowing				Days after sowing			
	35	50	65	Mean (V)	35	50	65	Mean (V)
KBC-2	731	612	630	658	453	565	519	513.0
V-322	740	634	663	679	506	456	409	457.0
GC-2	734	512	459	568	496	218	483	399.0
Mean (DAS)	735	586	584		485	413	470	
CD value at 5%	V = NS, DAS = 79, V × DAS = NS				V = NS, DAS = 72.70, V × DAS = 136.20			

a: average of four replications

Table 4. Relative abundance of ureides (RAU) in shoots and roots of cowpea varieties at different stages of growth.

Variety (V)	RAU in shoots[a] (%)				RAU in roots[a] (%)			
	Days after sowing (DAS)				Days after sowing (DAS)			
	35	50	65	Mean (V)	35	50	65	Mean (V)
KBC-2	75.5	66.8	61.3	67.8	87.5	63.3	58.8	69.9
V-322	87.0	76.8	69.8	77.9	78.7	63.9	64.5	69.1
GC-2	75.7	67.2	51.5	64.8	90.1	71.9	57.9	73.3
Mean (DAS)	79.4	70.3	60.9		85.5	66.4	60.4	
CD value at 5%	V = 3.7, DAS = 3.3, V × DAS = NS				V = 3.60, DAS = 3.80, V × DAS = 6.57			

a: average of four replications

The decrease in RAU may be also due to decreased ARA and Lb and increased nitrate-N content. Similar observations were also reported in soybean by Herridge (1982). A significant positive correlation between the ureide content with ARA and Lb content was reported in cowpea (Thimmaiah *et al.* 1999) and peanut (Peoples *et al.* 1986).

Nitrate-N and Total-N content: Table 5 shows the nitrate-N and total-N content in shoots of cowpea varieties at different DAS. The nitrate-N content increased whereas, the total-N content decreased linearly with an increase in DAS. The highest mean nitrate-N content was noticed in KBC-2 (306 µg/g dry w) and lowest in V-322 (204 µg/g dry w). Again, KBC-2 showed the highest mean total-N content (3.12%) and the lowest was in GC-2 (2.50%).

Herridge (1984) observed that the soybean plants accumulated very little amount of nitrate-N and it was found to decrease with an increase in the age of plants. However, increased level of nitrate-N at an early stage of growth had all been reported in soybean (Herridge 1982). The increase in total-N content at the early stages and decrease at later stages of growth were reported in *Cicer arietinum* and soybean (Matsumoto *et al.* 1977). A positive correlation between ARA and total-N content was also reported in bean (Wych and Rains, 1979) and soybean (Herridge, 1982: Herridge, 1984). However, an inverse relationship between nitrate-N and ARA and ureide content was noticed in soybean (Herridge 1982).

The study thus suggested that the nodule ARA, Lb content, shoot and root ureide, RAU and shoot total-N content decreased, whereas, shoot and root nitrate-N increased with an increase in growth stages in cowpea varieties. In general, the cowpea variety, KBC-2 was found to be superior with regard to symbiotic nitrogen fixation, which could be further exploited in breeding programmes.

Table 5. Nitrate-N and total-N content in shoots of cowpea at different stages of growth.

Variety (V)	Nitrate-N in shoots[a] (µg/g dry w)				Total-N in shoots[a] (µg/g dry w)			
	Days after sowing (DAS)				Days after sowing (DAS)			
	35	50	65	Mean (V)	35	50	65	Mean (V)
KBC-2	230	300	390	306	3.4	3.4	2.6	3.1
V-322	105	190	317	204	3.6	2.9	2.2	2.9
GC-2	235	245	430	303	3.3	1.8	2.3	2.5
Mean (DAS)	190	245	379		3.4	2.7	2.4	
CD value at 5%	V = 20.0, DAS = 24.0, V × DAS = 40.0				V = 0.3, DAS = 0.2, V × DAS = 0.4			

a: average of four replications

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