



# Hybrid Pigeonpea (ICPH 2740) Nodular Activity and Biological Nitrogen Fixation as Influenced by Agronomic Practices

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## ABSTRACT

**Background:** To explore the impact of agronomic practices on nodulation, nitrogenase activity and biological nitrogen fixation in hybrid pigeonpea, a field investigation was under taken at BW5 block ICRISAT development center (IDC), International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, during *kharif*, 2021 and 2022.

**Methods:** The seedlings were raised in portrays in advance to onset of monsoon during last week of May (28<sup>th</sup> May in 2021 *kharif* and 30<sup>th</sup> May in 2022 *kharif*) in both the years by filling portrays with cocopeat and vermicompost in 1:1 ratio. Transplanting of 24 days and 25 days old seedlings were done on 20<sup>th</sup> June in 2021 *kharif* and 23<sup>th</sup> June in 2022 *kharif*, respectively, on the same day of dibbling in the main field at 100 cm × 100 cm, 120 cm × 120 cm square geometry and 150 cm × 60 cm. Nutrient management treatments were imposed one week after sowing.

**Result:** Among the planting methods higher nodule number and nitrogenase activity with transplanting compared to dibbling. 120 cm × 120 cm found higher nodule number plant<sup>-1</sup> and nitrogenase activity but biological nitrogen fixation was higher with 100 cm × 100 cm ha<sup>-1</sup> basis. With respect on nutrient management practices, control performed well in terms of higher nitrogenase activity and biological nitrogen fixation and on par with integrated approach of 100% soil test based NPK + vermicompost + PSB + seed treatment with *Rhizobium*. So, with transplanting method of establishment, 100 × 100 cm square plant geometry and 100% soil test based NPK + vermicompost + PSB + seed treatment with *Rhizobium* was recommended agronomic practices for hybrid pigeonpea.

**Key words:** Acetylene reduction assay, Biological nitrogen fixation, Hybrid pigeonpea, Nitrogenase activity, Nutrient management.

## INTRODUCTION

Pigeonpea (*Cajanus cajan* L.) is the second most important pulse crop after chickpea in India. Pigeonpea is grown in an area of about 4.5 m ha with production of 3.3 MT in the country. The national productivity of pigeonpea is only 729 kg ha<sup>-1</sup> give comparison to world productivity (Directorate of Economics and Statistics, GOI 2022). In Telangana, it is cultivated in an area of 2.96 lakh ha with production of 1.91 lakh tones and productivity of 647 kg ha<sup>-1</sup> (Directorate of Economics and Statistics, GOI 2022).

In India more than 90 per cent of pigeonpea area is under rainfed conditions (Tiware and Namrata, 2020). Now a day's due monsoon aberrations farmers unable to sow the pigeonpea in correct window as a result the length of vegetative period reduced (Susithra *et al.*, 2019) so, to avoid this the concept of transplanting is came into picture. But with normal conventional method of crop geometry like 150 × 30 cm leads to severe interplant competition, improper expression and higher flower drops due to low ventilation (Pradeep *et al.*, 2018). Transplanting hybrid pigeonpea under square system the distance between rows and plants is same which facilitates good branching, pod and seed set when compared to varieties. Due to higher branching ability of hybrid under transplanting conditions, more yields were obtained compared to normal dibbling (Ramanjaneyulu *et al.*, 2017; Mallikarjun *et al.*, 2015).

Biological nitrogen fixation is a process of great environmental and economic importance, performed by

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*Rhizobium* that will reduce atmospheric nitrogen (N<sub>2</sub>) to ammonia (NH<sub>3</sub>) and when in symbiotic association with plants, they provide NH<sub>3</sub>, which is readily convertible to other forms, such as amides and ureides Rufini *et al.* (2016). Seed treatment with rhizobium before sowing in pigeonpea is suitable alternative for increasing yields. ICPH 2740 is the first commercial pigeonpea hybrid released in Telangana state as Mannemkonda kandi in 2015 and yield potential of

this hybrid is ranges from 2407-3652 kg ha<sup>-1</sup> (Saxena *et al.*, 2016). Among the different agronomic practices, suitable planting method, planting geometry and optimum nutrition is essential for enhancing productivity in hybrid pigeonpea.

## MATERIALS AND METHODS

### Experimental site

A field study entitled with “Hybrid pigeonpea (ICPH 2740) nodular activity and biological nitrogen fixation as influenced by Agronomic practices” was conducted at BW5 block ICRISAT development center (IDC). International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, during *kharif*, 2021 and 2022. The rainfall received during *kharif*, 2021 and 2022 experimental periods was 998.4 mm in 55 rainy days and 1000 mm in 53.1 rainy days respectively. Experimental soil is moderately alkaline in reaction with pH of 8.1 and EC of 1.94 dSm<sup>-1</sup>. The N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O and organic carbon content of soil was, low (231.1 kg ha<sup>-1</sup>), medium (29.8 kg ha<sup>-1</sup>) and high (350.5 kg ha<sup>-1</sup>) and low (0.42%), respectively.

### Treatment and experimental design

The experiment was laid out in split-split plot design with two planting methods in main plots *i.e.*, M<sub>1</sub>: Dibbling, M<sub>2</sub>: Transplanting, three crop geometries in sub-plots *i.e.*, S<sub>1</sub>: 100 cm × 100 cm (10,000 plants ha<sup>-1</sup>), S<sub>2</sub>: 120 cm × 120 cm (6,944 plants ha<sup>-1</sup>), S<sub>3</sub>: 150 cm × 60 cm (11,111 plants ha<sup>-1</sup>) (Normal transplanting) and five nutrient management practices in sub-sub plots *i.e.*, N<sub>1</sub>: Control, N<sub>2</sub>: 100% soil test-based (STB) NPK (25:37.5:8.5 kg ha<sup>-1</sup>), N<sub>3</sub>: 100% STB NPK + vermicompost + PSB + Seed treatment (ST) with *Rhizobium*, N<sub>4</sub>: 150 % STB NPK, N<sub>5</sub>: 150% STB NPK + vermicompost + PSB + ST with *Rhizobium*. The pigeonpea hybrid used for this investigation was ICPH 2740. The seedlings for transplanting were raised in pro trays and sown on 28<sup>th</sup> May in 2021 *kharif* and 30<sup>th</sup> May in 2022 *kharif*, respectively. Transplanting of 24 days and 25 days old seedlings were done on 20<sup>th</sup> June in 2021 *kharif* and 23<sup>th</sup> June in 2022 *kharif*, respectively, on the same day of dibbling in the main field. Vermicompost 5 t ha<sup>-1</sup>, PSB (Phosphorus solubilizing bacteria 5 kg ha<sup>-1</sup> added to vermicompost one week prior to application and seed treatment with *rhizobium* 500 g ha<sup>-1</sup> seed 2 hours before sowing. Based on soil nutrient status the recommendation was made such that, if nutrient status is low, 25% more dose on recommended dose, if it is high, 25% less than RDF and recommended dose is based on medium nutrient status.

### Data collection and analysis methods

The plant were uprooted carefully by digging around the plant by crowbar and the plants were washed under running tap in sieve and the nodules were counted and expressed in (No. plant<sup>-1</sup>) at active nodulation stages *i.e.* 30, 45 and 60 DAS/DAT. Nitrogenase reduces acetylene (C<sub>2</sub>H<sub>2</sub>) to ethylene (C<sub>2</sub>H<sub>4</sub>) and the ethylene formed can be very sensitively detected by gas chromatography. In this method detached

root with nodules enclosed in air tight glass jar (700 ml) and 10% gas inside replaced by pure acetylene. After 60 minutes incubation at room temperature, 0.5 ml of gas inside is sampled using a hypodermic syringe and concentration of ethylene gas is analysed by gas chromatography in acetylene reduction assay (ARA). The amount of ethylene formed will be proportional to nitrogenase activity. ARA is expressed μmol C<sub>2</sub>H<sub>4</sub> plant<sup>-1</sup> hr<sup>-1</sup>. By using this nitrogenase activity we can calculate biological nitrogen fixation (kg ha<sup>-1</sup>) by area under curve method (Hardy *et al.*, 1968). 3 is the theoretical conversion factor of 3 moles of acetylene reduced/mole of dinitrogen reduced (Peoples *et al.*, 2009).

### Statistical analysis

Data on nodule number and nodular activity were subjected to analysis of variance procedures as outlined for split-split plot design (Gomez and Gomez, 1984). Statistically significance was tested by F-value at p=0.05 (5%) level of probability and critical difference was worked out where ever the effect was significant. Treatment differences that were non-significant were denoted as NS.

## RESULTS AND DISCUSSION

### Number of root nodules plant<sup>-1</sup>

The effect of different agronomic practices on nodule number was studied during both years of experimentation (Table 1) and significant variation was found. Among the planting methods at 30 DAS/DAT significantly higher number of root nodule plant<sup>-1</sup> were recorded with transplanting (16.4) compared dibbling (12.4). Whereas, in planting geometry higher number of nodules plant<sup>-1</sup> were recorded with 120 × 120 cm (6944 plants ha<sup>-1</sup>), which was statistically on par with 100 × 100 cm (10,000 plants ha<sup>-1</sup>) at all the stages of crop and with respect to the nutrient management options, significantly higher number of root nodules were recorded with control at 30, 45 and 60 DAS/DAT and which was on par with 100 % STB NPK + 5 t ha<sup>-1</sup> + vermicompost enriched with PSB + *Rhizobium* seed treatment. These treatments followed by application of 150% soil test based NPK 5 t ha<sup>-1</sup> + vermicompost enriched with PSB + *Rhizobium* seed treatment and on par with 100% soil test based NPK. Similar trend was observed during both the years of experimentation. Lower nodule number was observed with 150 % soil test based NPK during both the years at all the stages. As pigeonpea is a leguminous crop it is able to fix the atmospheric nitrogen. Wherever, nutrient demand is more, the fixation ability increases to meet the required demand. This could be the reason for the higher number of root nodules formation in control plot and also influenced by integrated nutrient approaches (Mishra and Prasad, 2010). During the both years of experimentation the interaction effect of planting method, plant geometry and nutrient management practices were found to be non-significant with respect to the number of root nodules plant<sup>-1</sup> at 30, 45 and 60 DAS/DAT. As pigeonpea is a deep rooted legume it has ability to draw the fixed phosphorus from deeper layers of

soil by releasing certain root exudates and acids (Piscidic acid) but, the nitrogen requirement should be meet my biological nitrogen fixation (Singh *et al.*, 2016). So, the recommended dose of nitrogen is very less, the required nitrogen demand of crop should be met by biological nitrogen fixation (Tigga and Singh, 2019), but this nodulation activity again depends upon the many external and management factors (Kumar and Dart, 1987). For successful nodulation certain agronomic practices at field level *i.e.* addition of rhizobium inoculum through seed treatment may help in the formation of effective nodule Patil and Padmani (2007); Mishra and Prasad (2010) and Rufini *et al.* (2016).

#### Nodular activity (Nitrogenase enzyme activity $\mu\text{mol C}_2\text{H}_4 \text{ plant}^{-1} \text{ hr}^{-1}$ )

As pigeonpea is leguminous crop nodule function and biological nitrogen fixation depends upon the nitrogenase enzyme activity. Nitrogenase activity was studied through acetylene reduction assay in *kharif* 2021 and 2022 (Table 2) and found that, significantly higher nitrogenase activity was recorded with transplanting at 30 DAT ( $36.8 \mu\text{mol C}_2\text{H}_4 \text{ plant}^{-1} \text{ hr}^{-1}$ ) compared dibbling ( $23.9 \mu\text{mol C}_2\text{H}_4 \text{ plant}^{-1} \text{ hr}^{-1}$ ). In plant geometry, higher nodule nitrogenase activity  $\text{plant}^{-1}$  was recorded with  $120 \times 120 \text{ cm}$  ( $6944 \text{ plants ha}^{-1}$ ), which was statistically on par with  $100 \times 100 \text{ cm}$  ( $10,000 \text{ plants ha}^{-1}$ ). Among the nutrient management practices, significantly

higher nitrogenase activity was found with control, which was on par with integrated nutrient management approach (*i.e.* 100 % soil test based NPK + vermicompost  $5 \text{ t ha}^{-1}$  + vermicompost enriched with PSB + *Rhizobium* seed treatment). Lowest nitrogenase activity was found with 150% STB NPK during both the years experimentation. From 30 to 60 DAS/DAT there increase in nodular activity in all the treatments but, from 60 to 75 DAS nitrogenase activity was decreased in all the treatments. Interaction effect of planting methods, plant geometry and nutrient management practices was found to be non-significant. The nodule number is indirect indicators of nitrogenase activity, but the enzyme nitrogenase activity which is actually involved in the biological nitrogen fixation (Bidlack *et al.*, 2001) which is forerunner for meeting nitrogen requirement of the plant (Peoples *et al.*, 2009; Barber *et al.*, 1976).

#### Biological nitrogen fixation ( $\text{kg ha}^{-1}$ )

In transplanting due to early establishment and vigorous growth of seedlings, at 30 DAT more nitrogen was fixed biologically compared with dibbling (Table 3). With respect to the plant geometry,  $150 \times 60 \text{ cm}$  ( $11,111 \text{ plants ha}^{-1}$ ) which was found on par with  $100 \times 100 \text{ cm}$  ( $10,000 \text{ plants ha}^{-1}$ ). Significantly lower nitrogen was fixed with  $120 \times 120 \text{ cm}$  ( $6944 \text{ plants ha}^{-1}$ ) on hectare basis. Among the nutrient management practices, 100 % soil test based NPK +

**Table 1:** Number of root nodules  $\text{plant}^{-1}$  of hybrid pigeonpea as influenced by agronomic practices during *kharif*, season.

Treatment	30 DAS/DAT			45 DAS/DAT			60 DAS/DAT		
	2021	2022	Mean	2021	2022	Mean	2021	2022	Mean
<b>Planting method</b>									
M <sub>1</sub> : Dibbling	11.1	13.6	12.4	25.8	25.0	25.4	28.3	32.6	30.5
M <sub>2</sub> : Transplanting	14.7	18.1	16.4	36.3	39.7	38.0	23.3	23.3	23.3
SEm $\pm$	0.2	0.4	-	0.5	0.5	-	0.2	0.2	-
CD (P=0.05)	1.2	2.5	-	3.0	3.2	-	1.0	1.4	-
<b>Plant geometry</b>									
S <sub>1</sub> : $100 \times 100 \text{ cm}$	13.0	16.3	14.7	31.4	32.3	32.3	26.5	28.4	27.5
S <sub>2</sub> : $120 \times 120 \text{ cm}$	13.5	17.2	15.4	33.0	33.7	33.7	27.0	30.0	28.5
S <sub>3</sub> : $150 \times 60 \text{ cm}$	12.3	14.0	13.2	28.7	29.2	29.2	23.9	25.4	24.7
SEm $\pm$	0.2	0.3	-	0.4	0.5	-	0.4	0.5	-
CD (P=0.05)	0.6	1.0	-	1.4	1.5	-	1.2	1.7	-
<b>Nutrient management</b>									
N <sub>1</sub> : Control	15.0	17.8	16.4	38.6	39.4	39.9	33.4	34.0	33.7
N <sub>2</sub> : 100% STB RDF	12.2	15.3	13.8	28.1	27.8	26.8	22.4	24.8	23.6
N <sub>3</sub> : 100% STB RDF + vermicompost + PSB + <i>Rhizobium</i>	14.1	17.0	15.6	36.0	36.4	35.9	30.4	33.5	32.0
N <sub>4</sub> : 150% STB RDF	9.9	12.9	11.4	23.3	24.0	24.0	18.7	21.1	19.9
N <sub>5</sub> : 150% STB RDF + vermicompost + PSB + <i>Rhizobium</i>	13.3	16.1	14.7	30.3	31.5	32.5	25.1	26.4	25.8
SEm $\pm$	0.5	0.5	-	1.0	0.9	-	1.0	0.7	-
CD (P=0.05)	1.4	1.5	-	2.9	2.5	-	3.1	2.1	-

All 2-way and 3-way Interactions are Non-significant.

**Table 2:** Nitrogenase activity ( $\mu\text{mol C}_2\text{H}_4 \text{ plant}^{-1} \text{ hr}^{-1}$ ) of hybrid pigeonpea as influenced by agronomic practices during *kharif*, season.

Treatment	30 DAS/DAT			45 DAS/DAT			60 DAS/DAT			75 DAS/DAT		
	2021	2022	Mean	2021	2022	Mean	2021	2022	Mean	2021	2022	Mean
<b>Planting method</b>												
M <sub>1</sub> : Dibbling	24.2	23.6	23.9	38.5	37.0	37.8	40.8	37.8	39.3	22.4	20.1	21.3
M <sub>2</sub> : Transplanting	36.7	36.9	36.8	67.0	70.1	68.6	55.9	53.8	54.9	34.1	30.5	32.3
SEm±	0.8	1.0	-	1.2	0.9	-	1.6	2.1	-	1.1	0.9	-
CD (P=0.05)	4.9	6.1	-	7.2	5.5	-	9.7	12.7	-	6.7	5.4	-
<b>Plant geometry</b>												
S <sub>1</sub> : 100 × 100 cm	30.4	30.1	30.3	52.7	54.2	53.5	48.0	46.6	47.3	29.1	28.1	28.6
S <sub>2</sub> : 120 × 120 cm	31.0	30.5	30.8	53.6	55.8	54.7	49.7	47.1	48.4	32.0	32.4	32.2
S <sub>3</sub> : 150 × 60 cm	30.0	29.2	29.6	51.9	50.5	51.2	47.4	43.8	45.6	25.0	24.1	24.6
SEm±	0.4	0.5	-	2.1	1.8	-	1.9	1.4	-	1.2	0.8	-
CD (P=0.05)	NS	NS	-	NS	NS	-	NS	NS	-	7.3	4.9	-
<b>Nutrient management</b>												
N <sub>1</sub> : Control	41.1	42.8	42.0	76.0	72.7	74.4	72.9	64.2	68.6	56.1	51.6	53.9
N <sub>2</sub> : 100% STB RDF	26.3	24.9	25.6	44.2	43.9	44.1	37.6	32.3	35.0	21.4	14.7	18.1
N <sub>3</sub> : 100% STB RDF + vermicompost + PSB + <i>Rhizobium</i>	37.5	36.7	37.1	66.9	70.4	68.7	62.6	60.5	61.6	40.6	36.4	38.5
N <sub>4</sub> : 150% STB RDF	20.9	20.5	20.7	36.4	37.3	36.9	30.9	31.0	31.0	16.2	14.6	15.4
N <sub>5</sub> : 150% STB RDF + vermicompost + PSB + <i>Rhizobium</i>	26.5	24.8	25.7	40.0	43.3	41.7	37.9	41.1	39.5	18.3	21.7	20.0
SEm±	0.4	0.7	-	2.1	1.6	-	1.9	1.1	-	0.9	1.1	-
CD (P=0.05)	1.3	1.9	-	6.1	4.5	-	5.2	3.4	-	6.1	6.6	-

All 2-way and 3-way Interactions are Non-significant.

**Table 3:** Biological nitrogen fixation ( $\text{kg ha}^{-1}$ ) of hybrid pigeonpea as influenced by agronomic practices during *kharif*, season.

Treatment	30 DAS/DAT			45 DAS/DAT			60 DAS/DAT			75 DAS/DAT		
	2021	2022	Mean	2021	2022	Mean	2021	2022	Mean	2021	2022	Mean
<b>Planting method</b>												
M <sub>1</sub> : Dibbling	0.76	0.74	0.75	1.75	1.69	1.72	2.99	2.87	2.93	3.98	3.78	3.88
M <sub>2</sub> : Transplanting	1.15	1.16	1.16	2.78	2.84	2.81	4.71	4.79	4.75	6.13	6.11	6.12
SEm±	0.02	0.03	-	0.05	0.03	-	0.10	0.08	-	0.13	0.11	-
CD (P=0.05)	0.14	0.22	-	0.30	0.21	-	0.61	0.54	-	0.81	0.67	-
<b>Plant geometry</b>												
S <sub>1</sub> : 100 × 100 cm	1.02	1.01	1.02	2.42	2.43	2.43	4.11	4.12	4.12	5.40	5.38	5.39
S <sub>2</sub> : 120 × 120 cm	0.72	0.71	0.72	1.71	1.72	1.72	2.92	2.92	2.92	3.87	3.85	3.86
S <sub>3</sub> : 150 × 60 cm	1.12	1.09	1.11	2.65	2.58	2.62	4.50	4.34	4.42	5.85	5.61	5.73
SEm±	0.03	0.04	-	0.06	0.05	-	0.52	0.48	-	0.34	0.26	-
CD (P=0.05)	0.09	0.12	-	0.21	0.18	-	1.70	1.52	-	1.10	0.83	-
<b>Nutrient management</b>												
N <sub>1</sub> : Control	1.29	1.34	1.32	3.13	3.16	3.15	5.47	5.31	5.39	7.50	7.13	7.32
N <sub>2</sub> : 100% STB RDF	0.83	0.78	0.81	1.93	1.86	1.90	3.22	3.06	3.14	4.15	3.80	3.98
N <sub>3</sub> : 100% STB RDF + vermicompost + PSB + <i>Rhizobium</i>	1.18	1.15	1.17	2.82	2.84	2.83	4.85	4.89	4.87	6.47	6.41	6.44
N <sub>4</sub> : 150% STB RDF	0.66	0.64	0.65	1.56	1.55	1.56	2.61	2.63	2.62	3.35	3.34	3.35
N <sub>5</sub> : 150% STB RDF + vermicompost + PSB + <i>Rhizobium</i>	0.83	0.78	0.81	1.88	1.85	1.87	3.10	3.18	3.14	3.98	4.16	4.07
SEm±	0.04	0.05	-	0.04	0.05	-	0.12	0.09	-	0.41	0.38	-
CD (P=0.05)	0.12	0.14	-	0.11	0.16	-	0.43	0.27	-	1.15	1.08	-

All 2-way and 3-way Interactions are non-significant.

vermicompost 5 t ha<sup>-1</sup> + vermicompost enriched with PSB + *Rhizobium* seed treatment was recorded higher nitrogen fixation and was found on par with control. Lower nitrogen fixation was found with 150% soil test based NPK during *kharif* 2021, 2022. The average nitrogen was fixed about 6.12 kg ha<sup>-1</sup> in transplanting, 5.73 kg ha<sup>-1</sup> with 150 × 60 cm plant geometry (11,111 plants ha<sup>-1</sup>) and 7.32 and 6.44 kg ha<sup>-1</sup> with control and 100% soil test based NPK + vermicompost 5 t ha<sup>-1</sup> + vermicompost enriched with PSB + *Rhizobium* seed treatment respectively. The higher root proliferation with transplanting, wider plant geometry leads to elevated root exudates secretions (Mark *et al.*, 2009; Sekhon *et al.*, 2018) might increase the microbial population in rhizosphere and proportionate biological nitrogen fixation (Tilak *et al.*, 2006 and Dhaka *et al.*, 2020).

## CONCLUSION

Hybrid pigeonpea performed well under transplanting, 100 × 100 cm plant geometry and 100% soil test based NPK + vermicompost 5 t ha<sup>-1</sup> + vermicompost enriched with PSB + *Rhizobium* seed treatment among nutrient management practices in terms of number of root nodules, nitrogen fixation which results required nutrient demand of the plant can be met for the growth and development of the crop.

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## Conflict of interest

This article publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language.

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