



# Studies on Nodulation Diversity and Leg Haemoglobin Content of *Vigna Trilobata* (L.) Verde. Cultivars from Andhra Pradesh and Telangana States

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## Abstract

*Vigna trilobata* was one of the important forage crops cultivated regularly in South India. In Andhra Pradesh and Telangana this crop was raised regularly as either intercrop or on margins of the crop fields. The nodules from *V. trilobata* plants raised in earthen pots filled with soils from different districts of A. P and Telangana were collected for the morphological studies. The total number of nodules per plant ranged from 98 to 253. Maximum number of nodules per plant of more than 200 was recorded in plants from Guntur and Khammam district plants. The characteristic pink colored root nodules were recorded in the plants collected from Krishna district soils. Brown colored nodules with white stripes were recorded in the nodules collected from Vizag, East and West Godavari, Khammam and Krishna district soils. Majority of the nodules are either Globose or round in shape. The highest leg-haemoglobin content in the nodule was 520µg/g in plants collected from Khammam at 90 DAS.

**Keywords:** *Vigna Trilobata*; Root Nodules; Leghaemoglobin

## Introduction

The Genus *Vigna* encompasses nearly 100-150 species which are annual or perennial legumes [1,2]. *Vigna* is one of the major nodulating Genera in the family Leguminosae. Out of 150 species, only 41 were reported to be nodulated including *Vigna trilobata* [1]. *Vigna trilobata* commonly called as Pillipesara, African gram, Jungle mat bean, Mukni, was mainly cultivated as short term pasture and green manure crop in India, Pakistan, Indonesia and Sudan. In India, Andhra Pradesh is one of the states with highest production of forage crops like Pillipesara and sun hemp. *Vigna trilobata* is a wild species belonging to the sub genus *Ceretotropis* in the genus

*Vigna*. Whyte, et al. considered that *Vigna trilobata* is a wild ancestor of Moth bean (*V. acanitifolia*). *Vigna trilobata* can be distinguished from the morphologically similar *V. acanitifolia* by virtue of large oval stipules, the latter having small linear-lanceolate stipules, and from *Vigna radiata* var. *sublobata* in having small seeds, flowers, pods, and a very long peduncle. According to Marechal, et al. the morphology of *Vigna trilobata* is very similar to that of *V. radiata* var. *sublobata* (88.95 % similarity). In Andhra Pradesh this crop was raised regularly in all coastal districts and few Rayalaseema districts as short term forage crop after rains. *Vigna trilobata* is cultivated as fodder or cover crop in India. Some tribes in India collect wild *V. trilobata* and eat. Nodulation in *Vigna trilobata* was

first reported in Japan by Asia and in India by Raju in 1936 [1]. Among Nitrogen fixing Legumes, Rhizobium symbiosis was one of the most promising and the bacterial species of rhizobium complex are very important. However there were no reports on this important forage legume *V. trilobata* so far. For the first time we are reporting the root nodulation diversity and leghaemoglobin content of the *V. Trilobata*.

## Materials and Methods

Soil samples were collected from agricultural fields under the cultivation of *Vigna Trilobata* from all districts of Andhra Pradesh for nodulation studies. Seeds of *Vigna trilobata* were raised in earthen pots filled with various district soils and were maintained properly in the botanical garden of Acharya Nagarjuna University garden. Nodulation data was recorded at 10 days intervals from 30 to 110 days after sowing. Three plants were gently uprooted for studies on nodulation characteristics like size, shape, colour, number, distribution, fresh weight, dry weight and moisture content of each nodule. Leg haemoglobin content of the nodules was estimated, according to the method described by Tu, et al. [3]. Nodules were collected from roots, washed under running tap water, followed by surface treatment with 0.1 % Mercuric Chloride, 95 % alcohol and sterile water. Nodules were washed for atleast 10 times with sterile water to remove the traces of Mercuric chloride. The nodules were transferred in culture tube.

## Leghaemoglobin Content

Leg haemoglobin content was estimated according to the method described by Tu, et al. [3]. All the nodule samples were frozen before leg haemoglobin extraction. Three replicate were maintained for each treatment. 500mg to 1g nodules were homogenized in 5ml of 0.1N KOH and centrifuged for 10 min at 12000 rpm. From this 1.5 ml of supernatant and to 1.5 ml of supernatant, 1ml of distilled water and 0.5 ml of 5 N KOH, 0.1g of  $\text{Na}_2\text{S}_2\text{O}_4$  were added for reduction and OD for leghaemoglobin content was measured at 537, 557, and 557 nm wave lengths by using Spectrophotometer. The Leg haemoglobin content was calculated using the formula

$$-\mu\text{g of leghaemoglobin} = \text{OD}_{557} - 1/2(\text{OD}_{537} - \text{OD}_{577}).$$

## Results and Discussion

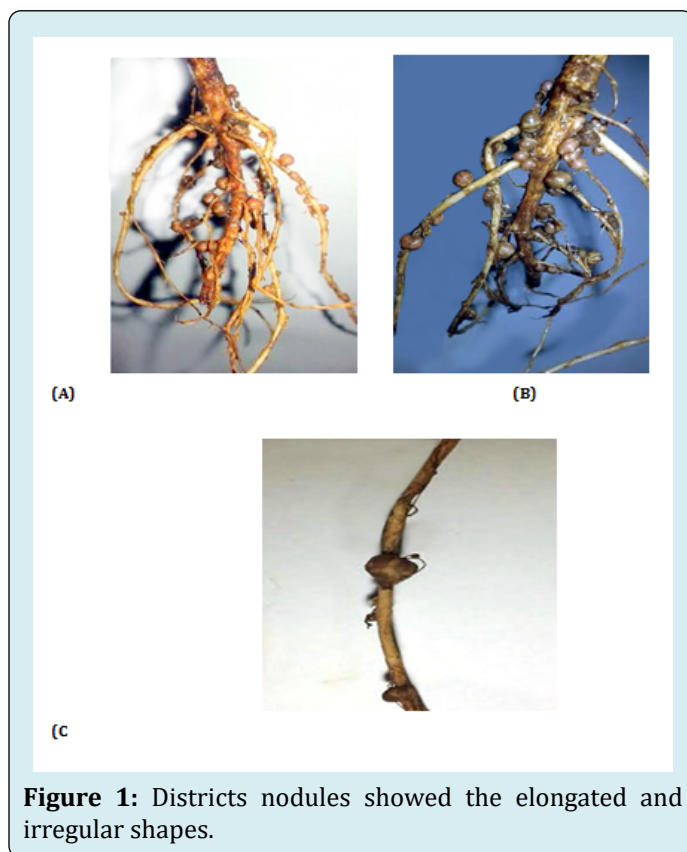
Nodulation data was collected at every 10 days interval. All districts of root nodules showed much variation. Nodulation was initiated much earlier at 21 DAS. Nodulation was initiated 3 weeks (21 DAS) after seed germination and the number was increased with age of the plant upto 90 DAS. Nodules were mostly observed on Tap root and as well as Lateral roots. Number nodules on Lateral roots are more

than Tap roots. Similar pattern of nodulation was reported by Anegbah, et al. [4] in *Indigofera zollingeriana* and by Bhaduri and Ratna Sen in *Phaseolous*. In majority of the districts nodule initiation was seen after 3 weeks (21 DAS), but in few districts like Srikakulam, Rangareddy, Kadapa nodule initiation was observed after 4 weeks (28DAS). Khammam district root nodules produced highest number (233) of nodules per plant at 90 DAS, Arya and Singh [5]. Lowest numbers of nodules were observed in Krishna district soils and the remaining districts soils show the moderate number of nodules per plant.

Majority of the districts nodules are either Globose or round in shape, where as Kurnool and Nizamabad districts nodules showed the elongated and irregular in shape.

A & B) Root nodulation pattern of *Vigna trilobata*

C) Nodules of brown colour with stripes from Prakasam Cultivars (Figure 1)



**Figure 1:** Districts nodules showed the elongated and irregular shapes.

The characteristic pink coloured root nodules were recorded in the plants collected from Krishna district soils. Brown coloured nodules with white stripes were recorded in the nodules collected from Vizag, East and west Godavari and Prakhasam districts. Brown coloured nodules were observed in the soils of Nizamabad, Guntur, Anapatur, later the colour of the nodule changed from brown to pink. Nodules were mostly brown or pink indicating their effectiveness in

nitrogen fixation. In all district plants, nodule size ranges from 1mm to 5 mm in diameter. In the districts of Nellore, Prakhasam, Krishna district nodules shows maximum size of 5 mm, where as in the districts of Anantapuram, Warangal, and Hyderabad nodule size is 3mm in diameter.

Fresh weight of the each nodule was maximum with 0.59 mg in Nellore district soil and lowest fresh weight was 0.18 mg in East Godavari district soils nodule. The dry weight of each nodule was maximum of 0.31 mg in Nellore and

Prakhasam districts, and the lowest in dry weight of 0.09 mg in the nodules of Krishna district. The leg haemoglobin content of the nodules gradually increased with age of the plant from 30 DAS and reached maximum of 520 µg/g was observed in soils of Krishna district soil. Increased leghaemoglobin content of the nodules reached at 90 DAS. Similar type of positive correlation was also reported by Sindhu, et al. [6] in 6 legumes and Subba rao and Chopra [7] in Soybean, Raghava, et al. [8] (Table 1).

S.No	Name of the District	leghaemo-globin	Number of Nodules/ plants at 90 DAS			Size	Shape	Colour	Weight of the nodule(mg)	
			Tap Root	Lateral Roots	Total				Fresh	dry
1	Srikakulam	342	49	76	125	4-Jan	Round	Brown	0.19	0.09
2	Viziayanagaram	389	36	94	130	4-Jan	Globose	Pink	0.21	0.09
3	Visakhapatnam	380	26	102	132	4-Jan	Round	Brown colour with white stripes	0.29	0.12
4	East Godavari	312	40	65	105	3-Jan	Round	Brown colour with white stripes	0.18	0.09
5	West Godavari	408	35	70	105	4-Jan	Globose	Brown colour with white stripes	0.19	0.1
6	Krishna	521	25	73	98	4-Jan	Globose	Pink	0.21	0.05
7	Guntur	409	30	175	205	4-Jan	Globose	Brown to pink	0.57	0.15
8	Prakasam	312	59	143	202	5-Jan	Round	Brown colour with white stripes	0.57	0.31
9	Nellore	428	43	210	253	5-Jan	Round	Pink	0.59	0.31
10	Chittoor	467	50	127	177	4-Jan	Round	Brown colour with white stripes	0.49	0.28
11	Kadapa	420	29	110	139	4-Jan	Globose	Cream	0.45	0.23
12	Kurnool	456	32	110	142	4-Jan	Elongated	Pink	0.4	0.23
13	Anantapuram	468	35	93	128	3-Jan	Round	Brown to pink	0.39	0.15
14	Medak	368	30	85	115	4-Jan	Round	Light green white stripes	0.35	0.18
15	Ranga Reddy	392	33	73	106	4-Jan	Round	Brown	0.25	0.1
16	Warangal	332	30	71	101	3-Jan	Round	Brown	0.24	0.13
17	Khammam	332	35	70	105	4-Jan	Round	Brown to pink	0.29	0.12
18	Nizamabad	444	40	93	133	4-Jan	Irregular	Brown to Pink	0.39	0.14
19	Mehaboob Nagar	469	41	70	111	4-Jan	Round	Brown to pink	0.42	0.28
20	Nalgonda	481	31	76	107	4-Jan	Round	Brown	0.31	0.18
21	Acharya Nagarjuna University	401	26	72	98	3-Jan	Round	Brown colour with white stripes	0.35	0.15
22	Hyderabad	407	19	71	90	3-Jan	Round	Brown	0.34	0.18
23	Karim Nagar	403	21	83	104	4-Jan	Round	Brown	0.39	0.18
24	Khammam	520	52	181	233	5-Jan	Round	Brown	0.57	0.15
25	Adilabad	412	20	80	100	4-Jan	Irregular	Brown	0.39	0.15

**Table 1:** Comparative account on Root Nodule characters of *Vigna trilobata* plants raised in all districts soils of Andhra Pradesh. \*Each data is an average of three replicates

## Discussion

The nodulation characteristics of *Vigna trilobata* cultivars including number, distribution of nodules, size, shape, color, fresh weight and dry weight of nodule were recorded at regular intervals from 40 to 90 days after sowing. All the 25 cultivars of *V. trilobata* showed much variation in nodulation characteristics. The nodules were distributed both on tap root and lateral roots. In general more number of nodules was present on taproot Kahn, while many more nodules were formed on “secondary roots” of cowpea. There is a evidence in our research the largest nodules from the cultivars, the isolated rhizobia produced maximum amount of exopolysaccharides and phosphate solubilizers Kumar GK and Ram MR [9,10].

The root nodules of *V. trilobata* are round to oval type at the early stage but become elongated with increase in age. Among the cultivars studied the average of nodules/plant was more (253 nodules/plant) at 90 DAS, was recorded in cultivars. The number of nodules per plant was 98 and 90 in the cultivars from Krishna and Hyderabad soils respectively. However, much low number of nodules per plant in the range of 22 - 47 was reported in *V. trilobata* when inoculated with different Australian *Rhizobial* strains [11]. Sharma, et al. [12] studied the nodulation and fresh weight of nodules/plant in *V. radiate* [13,14].

## Conclusion

The present study reveals that the nodulation characteristics like number of nodules, size, colour and shape of the nodules were varied from cultivar to cultivar. Maximum number and size of the nodules were represented for further studies like isolation of rhizobium and its characterization.

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