Table 1. Per cent out-crossing in okra CV 'Pusa Sawani'

Distance (m)	Per cent out-crossing					
	Trial I			Trial II		
	PI	PII	PIII	PI	PII	PIII
10	3.5	0.0	0.0	1.3	2.4	2.1
20	6.7	0.0	0.0	1.2	0.5	1.8
30	3.4	0.0	0.0	1.0	0.0	1.5
40	0.0	0.0	0.0	0.0	0.0	0.0

(Thakur and Arora, 1986). In the present study, all the plants from the seeds of direct and reciprocal crosses of 'Pusa Sawani' x 'CO 1' revealed red pigmentation confirming that this colour is dominant to green colour and can act as a market in the isolation experiment.

Okra is potentially a self-pollinated crop. Because of its showy corolla, the possibility of cross pollination by insects cannot be ruled out. Consequently cross pollination to the extent of 4.0 to 42.2 per cent has been reported (Thakur and Arora 1986). In the present study, percentage out crossing varied from 0.5 to 6.7 depending upon the distance of the contaminating source and picking (Table 1). In trial I, crossing was noticed only in first picking seeds; whereas, in trial II crossing was observed in all three pickings. In both the trials, no crossing could be recorded beyond 30m isolation provided. Hence, it became evident that for okra seed production, the isolation distance requirement can be reduced to 30m for certified seed and 50 m for foundation seed class (providing an additional safeguard of 20m). For cotton, (*Gossypium histutum* L), the recommended isolation distances are 30 and 50m for certified and foundation seed classes, respectively. It will be interesting to note that both okra and cotton belong to the same family, Malvaceae, with similar floral structures.

Second generation seeds of the first trial did not give any pigmented plant. It was expected since all the pigmented plants from first generation seeds were removed before flowering and more so this character was governed by a single dominant gene.

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CHARACTERISTICS OF ROOTS AND NODULATION IN LEGUMES

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ABSTRACT

A comparative study on the characteristics of root system was made between certain profusely nodulating and non-nodulating (21 legumes; 14 genera) to assess the influence of various factors on nodulation. There existed no relation between root hair or thinness of the root or reduction in the cortex and nodulation in these legumes. The low or high levels of sugars, total phenols and aminoacids in roots also had no relation to the nodulation of legumes. The results indicated that the factors tested in this investigation may not be the sole reasons for non nodulation of certain legumes.

Leguminous species exhibit a wide variation in nodulation (Lim and Burton, 1982). Nodulation is common in Papilionoideae and Mimosoideae plants while about 70 per cent of Caesalpinoideae lack nodulation (Allen and Allen, 1961). Based on the nodulation profile of the genus *Cassia* of Caesalpinoideae it was concluded that the absence of nodulation was due to morphological factors like absence of root hairs, meagre production of root lets, reduced cortex, presence of antibacterial compounds like phenols and immobilization of rhizobial cells even if they enter into the root system (Allen and Allen, 1976). In the present investigation a comparative study was made on the characteristics of root system viz., colour, thickness of roots and cortex and the contents of sugars, aminoacids and phenolic compounds in selected nodulating and non-nodulating legume species and the results are presented here.

Table 1. Nodulation and nitrogenase activity of legume species

Legume species	Nodule no/plant	Nodule dry weight/ plant (mg)	Nitrogenase activity*
Cassia fistula L.	-	-	-
C.occidentalis L.	-	-	-
C.grandis L.f.	-	-	-
Erythrina indica Lam.	30.3	190.0	98.5
Sesbania aculeata (Pers)	7.3	17.1	9.6
S.grandiflora (L) (Pers)	23.8	132.0	39.7
S.rostrata Brem.	24.4	10.0	50.6
Acacia nilotica (L.) Willd	18.5	20.0	-
A.mellifera (L.)	0.8	2.5	-
A.auriculiformis A. Cunn ex.Benth	9.4	12.0	42.6
Abrus precatarius L.	1.6	10.0	-
Crotalaria juncea L.	9.9	16.1	8.2
Tamarindus indica L.	-	-	-
Delonix regia (Boj) Raf	-	-	-
Leucaena leucocephala (Lam) Dewit	8.7	6.0	27.5
Peltophorum ferrugenum Baker ex. Heyne	-	-	-
Vigna radiata ** (L.) Wilczak	10.0	8.0	-
*V.unquiculata*** (L.) Walp	16.2	18.0	132.8
Cajanus cajan ** (L.) Millsp.	21.2	66.0	31.4
Mimosa pudica (L.)	6.7	10.0	-
Prosopis juliflora (SW.) DC.	8.2	22.0	25.6

* nm of C_2H_2 reduced/h/g nodule dry weight

** No acid treatment and soaking

MATERIALS AND METHODS

Seeds of the test legumes were pretreated with concentrated sulphuric acid for 2-3 minutes and washed in several changes of water, soaked for 6 h and sown without any bacterization in unsterilized field soil previously cultivated with sunnhemp (*Crotalaria juncea* L.). Grain legumes like green gram (*Vigna radiata* (L.) Wilczek), cowpea (*V.unguiculata* (L.) Walp.) and pigeonpea (*Cajanus cajan* L. Millsp.) were also sown without acid treatment or soaking. After 30 d ten plants were uprooted with least injury to roots and examined. The colour of the root was recorded visually. The girth of the root was measured in two planes 1cm below the collar region using a verniar calipers. The cortex thickness was measured at 3 or 4 planes in thin section of roots by micrometry and the average was determined. The root hairs were observed in seedlings germinated in petridishes on 2nd and 5th d of germination by examining the radicles in Zoom Nikon microscope (Nippon Kogaku KK, Japan). (Magnification - 50 times).

The nitrogenase activity was determined by acetylene reduction assay (Somasegaran and Hoben, 1985) in Vista Varian Gas Chromatograph Varian Vistogok series 5000 attached to a computarised terminal using Porapak column 80-100 mesh and FID with an ignition temperature of 150° C. The roots with undetached nodules were washed free of adhering soil, blotted with filter paper, transferred to serum vials and closed air tight with butyl rubber stoppers. Ten ml of air was evacuated from the vials and 10 ml of acetylene was injected. After 1 h incubation at 28 ± 2° C, 1 ml of the gas was withdrawn and injected into the gas chromotagraph. The nodule number also counted and the nodule and plant dry weights were determined by drying them at 105° C for 24 h. The reducing and total sugars, amino nitrogen and total phenolic compounds in the roots were determined in the ethanol extracts of roots following the standard methods (Mahadevan and Sridhar, 1986).

RESULTS AND DISCUSSION

Plants with white coloured roots nodulated profusely compared to those having coloured roots. The light brown or creamy roots exhibited moderate nodulation. The legume species belong to Papilionaceae *viz., Erythrina indica* Lam., *Sesbania aculeata* (Pers.), *S.grandiflora* (L.) Pers., *S.rostrata* Brem, *C.juncea* L., *V.unguiculata*, *C.cajan* L. Millsp. and species belong to Mimosaceae viz., *Acacia* spp., *Leucaena leucocephala* (Lam.) Dewit., nodulated well. The roots of these plants were white coloured. The dark brown coloured roots of Caesalpiniaceae species *viz.. Cassia occidentalis* L., *C.grandis* L. f., *C.fistula* L., *Delonix regia* (Boj.) Ref. and *Tamarindus indica* L. failed to show any nodulation. The nitrogenase

Table 2. Physical characteristics of legume species

Legume species	Root colour	Root hair	Root girth (mm)	Cortex thickness (mm)
Cassia fistula L.	Dark brown	-	1.5	0.27
C.occidentalis L.	Dark brown	Profuse	1.3	0.18
C.grandis L.f.	Dark brown	Moderate	2.8	0.63
Erythrina indica Lam.	White	Sparse	4.4	0.50
Sesbania aculeata (Pers)	White	Profuse	1.6	0.29
S.grandiflora (L) (Pers)	White	Sparse	2.6	0.59
S.rostrata Brem.	White	Profuse	2.1	0.28
Acacia nilotica (L.) Willd	White	Sparse	1.3	0.22
A.mellifera (L.)	White	Sparse	1.9	0.27
A.auriculiformis A. Cunn ex.Benth	White	-	0.7	0.17
Abrus precatarius L.	Light brown	-	1.8	0.39
Crotalaria juncea L.	White	Profuse	2.3	0.47
Tamarindus indica L.	Dark brown	Sparse	2.1	0.28
Delonix regia (Boj) Raf	Dark brown	-	2.5	0.22
Leucaena leucocephala (Lam) Dewit	White	Moderate	2.9	0.24
Peltophorum ferrugenum Baker ex. Heyne	White	-	1.8	0.21
Vigna radiata ** (L.) Wilczak	White	Profuse	1.5	0.19
*V.unquiculata*** (L.) Walp	White	Profuse	1.9	0.08
Cajanus cajan ** (L.) Millsp.	White	Profuse	1.3	0.08
Mimosa pudica (L.)	White	-	0.8	0.08
Prosopis juliflora (SW.) DC.	White	Sparse	0.9	0.13

activity was the highest in cowpea followed by *E.indica* and *A.auriculiformis* with 132.7, 98.5 and 42.6 nm of C_2H_2 reduced/h/ g nodule dry weight. There was no detectable nitrogenase activity with *Abrus*, *A.nilotica*. Non-nodulated plants failed to show nitrogenase activity. (Table 1).

Among the legumes tested plants with a relatively thicker root system like *E.indica* (4.4 mm), *S.grandiflora* (2.6mm) and *C.juncea* (2.3 mm) recorded a higher nodulation in general. However, *D.regia* with a thicker root failed to nodulate naturally or after artificial bacterization despite its equal thickness of root system with the afore-mentioned legume species. However, species like *S.aculeata* with medium thick roots (1.6 mm), *V.unguiculata* (1.9 mm) and *V. ratiata* (1.4 mm) recorded good nodulation. *Acacia nilotica (L.) and Willd. ex Del (1.2 mm) and Abrus precatorius* L. (Papilionaceae) (1.8mm) with their wiry thin roots nodulated. However, the thin wiry root of *C.fistula* and *C.occidentalis* failed to show any nodulation. Although the number of nodules increased with the thickness of the root system in nodulating legume (Anthoniraj *et al.* 1986), the thickness of the root *per se* might not contribute to the presence or absence of nodulation in the legume species as the plants with thicker roots like *D.regia* and *C.grandis* totally failed to exhibit any nodulation.

The presence of nodulation in plants with meagre cortex region like cowpea (0.079 mm), *Mimosa pudica* L. (Mimosaceae) (0.081 mm), *C.cajan* (0.084 mm), *Acacia auriculiformis* A. cunn ex. Benth (0.169 mm) (Table.2) and the absence of nodulation with moderately extended cortex in *D.regia* (0.223 mm) *T.indica (0.281mm) and with largely extended cortex region as in C.grandis* (0.631mm) suggest that the meagre or extended cortical system region might not influence the nodulation characteristics of a legume. The presence of poor or abundant root hairs also do not determine the extent of nodulation as *C.occidentalis* and *C.grandis* with profuse root hairs did not nodulated at all.

The biochemical analyses of the roots showed that low or high levels of either sugars or amino acids might not determine the nodulation (Table 3). The roots with high and low sugar contents were free from any nodulation. The species of *Cassia* viz., *C.grandis* having a high sugar content and *C.occidentalis* or *C.fistula* with low sugar content

Table 3. Chemical characteristics of legume species

Legume species	Sugars Total (mg/g)	Sugars Reducing (mg/g)	Amino Nitrogen (mg/g)	Total Phenols (mg/g)
Cassia fistula L.	16.543	6.617	5.046	11.860
C.occidentalis L.	7.343	5.727	2.103	8.360
C.grandis L.f.	14.139	13.091	5.179	6.376
Erythrina indica Lam.	8.969	6.784	3.354	3.297
Sesbania aculeata (Pers)	13.108	5.433	1.735	6.183
S.grandiflora (L) (Pers)	9.813	4.018	1.197	2.881
S.rostrata Brem.	12.752	15.129	1.534	5.630
Acacia nilotica (L.) Willd	19.580	6.110	1.216	7.930
A.mellifera (L.)	21.070	10.343	4.611	1.990
A.auriculiformis A. Cunn ex.Benth	18.197	14.558	7.800	!8.260
Abrus precatarius L.	11.524	4.195	4.044	2.256
Crotalaria juncea L.	11.612	5.465	1.946	3.761
Tamarindus indica L.	8.179	6.523	6.740	5.850
Delonix regia (Boj) Raf	10.539	6.183	2.595	7.888
Leucaena leucocephala (Lam) Dewit	23.084	4.991	3.840	6.561
Peltophorum ferrugenum Baker ex. Heyne	18.349	4.094	3.200	5.654
Vigna radiata ** (L.) Wilczak	19.310	6.313	2.448	3.195
*V.unguiculata*** (L.) Walp	11.535	8.552	1.967	5.987
Cajanus cajan ** (L.) Millsp.	13.900	7.329	2.333	2.356
Mimosa pudica (L.)	36.390	2.400	2.870	2.400
Prosopis juliflora (SW.) DC.	25.196	16.797	1.385	10.026

in roots were devoid of any nodulation. However, the roots of *E.indica* with low sugar content facilitated fairly good nodulation. The low or high sugar levels in the root system of leguminous plants might not be a significant factor in nodulation as both low and high levels of sugars were exhibited by non-nodulating legumes and roots with low levels favoured good nodulation. The roots tissues of *A.auriculiformis, A.nilotica, S.aculeata* and *Leucaena leucocephala* with a higher quantity of total phenols exhibited profuse nodulation while *C.occidentalis* with lower content of total phenolic compounds showed no nodulation suggesting that the status of total phenolic compounds might not be an inhibitory factor for nodulation although certain specific phenolic compounds might exhibit antibacterial action. It is likely that the nature and type of phenolic compounds may affect the nodula tion and not their total levels as in the case of disease resistance against pathogen (Mahadevan, 1973).

The nodulation was absent in *C.fistula* and *T.indica* having high levels of free amino acids in their roots while abudant nodulation was observed in *S.grandiflora* and *S.aculeata, V.unguiculata* and *C.juncea* with lower levels of amino acids. *A.precatorius* with relatively higher levels of amino acids also exhibiting nodulation depicting that no concrete relationship of amino acid levels with nodulation.

The results clearly revealed that the thickness of root or cortex or the levels of sugars, amino acids or phenolic compounds in the root system are not the determinant of nodulation.

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