Dynamic studies of Nodulation and morphological Characterization of *Dhaincha*(*Sesbania aculeate* L.) accessions

Abstract

Sesbania aculeate is a quick growing, succulent, easily decomposable and produces maximum amount of organic matter and nitrogen in the soil. Sesbania green manure after decomposition, increases humus, available nitrogen and lower down the C:N ratio of soil, aeration, permeability, enhance the boron and iron content in soil and also protect the soil from leaching of nutrients. Besides this significance, current study was carried out during *rabi* 2022 at ADAC&RI, Trichy to characterize the explored daincha germplasms for their morpho biometrics triats, green biomass yield and nodulation. Daincha germplasm Sivagangai local recorded more green biomass yield(19.8t/ha) and superior contributing biometricaltraits.The number of Rhizobium colonies was higher in Sivagangai local as compared to Villupuram local, Vellore local & Pant variety. This could further be utilized for their symbiotic effectiveness determination under field condition and can be effectively utilized in hybridization programs for improving more green biomass yield.

Keywords: *Sesbania aculeate* L., Dhaincha, biomass, soil fertility, green manuring, nodules

Introduction

Sesbania aculeate L is a fast growing green manure crops has higher fresh biomass and produces maximum amount of organic matter and nitrogen in the soil(Palaniappan and Siddeswaran , 2001.). To lesser extents they are grown for animal feed and fodder (Kabir, A.K 2018 ; Heering, J.H.,1996 a), ground cover, providing wood, firewood and other uses in traditional agroforesty systems (Evans, D.O. and Rotar, E.P. 1987.). It grows well even in marginal lands with little or no input. It showed a luxuriant growth in soil with a high electrical conductivity up to 10 mS cm-1, and some of the *Sesbania*spp. have been recommended for reclamation of saline and sodic soils (Bisht, P. S., 2006). Long time (10/12 yrs) cultivation of *dhaincha*would combat desertification of marginal lands, e.g., char land, saline area, etc., and rehabilitate degraded lands into productive crop lands for intensive food crop agriculture (Bhander, P. K., 1998). Among green manuring crops, *Sesbaniaaculeate* L. is one of the most important crops and itsincorporation in the soil adds about 60-80 kg nitrogen/ha (Beri, V 1989)

and improves soil structure, aeration,permeability and also protect the soil from leaching of nutrients, also enhance the boron and iron content in soil.Decomposed materials of sesbania also serve as chelating compound and helps in increasing the availability of nutrients i.e. Zn, Cu, Mn etc. in succeeding crop (Onim, J.F.M., *et al. 1990*)

*Dhainchaa*lso showed its potentiality as a raw material for paper pulp (Jahan *et al*., 2009). Researchers, recently, have found that the leaves of *dhainchaa*re a good source of Pinitol, an anti-diabetic compound (Milan, M. A., 1985).Nitrogen is an essential nutrient for plant growth and its replenished *via* Nitrogen fertilizer or biological nitrogen fixation. Rhizobia are the symbiotic nitrogen fixers and form nodules on the roots of legume plants. The plant supplies the rhizobia with energy in the form of amino acids and the rhizobia fix nitrogen from the atmosphere for plant uptake. Nodules are the special structure produced by rhizobia in legumes; the nodule size (small to medium) and the morphology of the nodules vary (round, oval or irregular shape) with the plant species.

Materials and methods

The experiment was carried out at AnbilDharmalingam Agricultural college and Research Institute, Tiruchirapalli, Tamil Nadu Agricultural University, Tamil Nadu, India, during late *Rabi* 2022. Four *Dhainchaa*ccessions, Sivagangai local, Villupuram local, Vellore local and Pant used for the current study (Table 1).The experiment was laid out in Randomized Block Design with four replications. The plot size was 4.0 x 2.7 m with spacing of 45 x 20 cm. All the recommended packages of practices were adopted besides providing necessary prophylactic plant protection measures to raise a good crop.The observations on Plant Height at 45 days(cm), Plant height at maturity(cm), Number of Branches per plant, Number of pods per plant, Number of pods per cluster, Pod length(cm), Number of seeds per pod, 1000 seeds weight(g), Fresh weight per plant (g), Green biomass(t/ha), No. of root nodules/plant , plant population/m^2 wererecorded. Experimental data were collected 45 days after sowing (DAS) at the seedling to maturity stages (80% pod attained to characteristic colour). Thirty plants per plot were used for data collection. Base diameter was measured at 5cm above the ground level. For biomass yield, seedlings were oven dried at 72±20 C for at least 24 hrs, mature plants were sun dried properly. Botanical identification based on morphological descriptors was done at the Plant Systematics Laboratory of the same Department following standard taxonomic procedures (Becker, M., 1995).

Nodulation studies

Five plants from each accession were collected. Dhaincha plants were uprooted carefully. Detached root nodules were washed in tap water to remove the adhered soil particles. The number of root nodules was counted.

Isolation of root nodulating bacteria

A total of four plants were recorded for assessment of Rhizobium in root nodules. For rhizobium assessment, root nodules were collected from young and healthy plants of all four daincha cultures.Healthy Dhaincha root nodules were detached from root and further isolation of root nodulating rhizobia was carried out. The detached root nodules were washed in tap water to remove the adhere soil particles from the nodule surface.Then,the detached root nodules are washed with 70% ethanol and rinsed with water.Mercuric chloride was used to treat the nodules and nodules are not wash with water. At the end of this process nodules are transformed from brownish to creamy white colour. Then the surface sterilized root nodules were transferred in test tube containing 10mlsterilized distilled water.These nodules were crushed with the help of sterilized glass rod to obtain in milky suspension of bacteriodes.Serial dilution was made upto 10^{-5}. From the 10^{-5} for each cultivar 3 replication plates were used. For Rhizobium assessment YEMA medium was used. Pour plate method was followed. The plates were sealed by parafilm to avoid contamination and incubated at 28 ±1˚C for 24- 48 hours. Rhizobium colonies were remained white translucent, elevated,mucilaginous, gummy raised colonies formed after 24± 72 hours.

GRAM STAINING

Bacterial smear of different strains was prepared separately and fixed in flame. Smears were fixed by passing over a Bunsen burner flame and then stained with Ammonium oxalate crystal violet for one minute. Then, it was washed with tap water and immersed in Gram's iodine for one minute. Again, washed with tap water and blot dried smear was flooded with 95% ethyl alcohol (decolorize) for 30 seconds.

It was again washed with water and blot dried carefully. Then it was counter stained with safranin, again washed with tap water and finally dried and examined under oil immersion objective on the microscope. Gram – negative bacteria retain the pink/red colour while Gram – positive bacteria retain the crystal-violet.

CONFIRMATORY TEST

CATALASE TEST

This test was performed to study the presence of catalase enzyme in bacterial colonies *rhizobium colonies* (24 hours old) were taken on glass slides and one drop of hydrogen peroxide (30%) was added. Appearance of gas bubble indicated the presence of

catalase enzyme(Mann, R. A 2000).In this test we observed gas bubbles so that we confirmed the raised bacterium is rhizobium.

Results and DISCUSSION

Various green manuring traits of *Sesbania aculeate* L. *viz*.,plant height, number of root nodules, green biomass, dry weight of biomass and visual observations were recorded before incorporation at 45 DAS.A total of four accessions of *dhaincha*germplasms, have been collected from different parts of of Tamil Nadu (Table 1). Morphological descriptors were collected and tabulated all the accessions.(Table 3) The *Sesbania* accessions showed a relatively wider variation in floral and fruit morphological descriptors, which might be due to natural hybrid nature of these accessions. The biometicaltriats were observed and tabulated in Table2.

Among the accessions Sivagangai local recorded the more plant height (158 cm) followed by Villupuram local(137 cm) and Vellore local(130 cm) . The greater number of root nodules were observed in Sivagangai local (52.4). followed by Vellore local and pant variety showed the lowest value of (40.4). Green Biomass yield varied from 11.3 ton to 19.8 ton /ha, where highest value was noted in Sivagangai local (19.8 ton per hectare) and the lowest value was recorded in pant variety (11.3 ton per hectare). The average plant population in Sivagangai local, Villupuram local, Vellore local and Pant are 400 plants/m^2,350 plants/m^2,320 plants/m^2 and 290 plants/m^2 respectively (Table 2).Green Biomass yield and yield contributing distributors also varied due to the genetic makeup of dhaincha accessions (Jahan, M.S., 2009). Yield contributing factors did not totally dependent upon the performance of individual plant but also on plant population per unit area.The plant height, leaflets per leaf,dryweight of plant have positive direct effect on biomass yield and used as selection criteria for the improvement of dhaincha biomass yield.

The canopy of the crop would be bushy at early vegetative stage and result in green biomass yield might be increased. The growth duration and the leaf area determine the overall of solar radiation intercepted by the canopy, as well as influences the amount of photosynthesis, finally biomass production (Abbro , M.B. and Abbasi, Z.A.(2002).).

Morphological descriptions

Botanical identification based morphological characters are tabulated in Table 3/Fig.4 Tested accessions were erect and determinate growth habit. The time of 50% flowering ranged from 42 to 55 days. The stem is glaborous ,Sivagangai local have Green with red long

stripes, Villupuram local have Green with brown strips. The stem is pubescent in the early period latter non pubescence. The leaf length varying from 7-25 cm and the no. of leaflets varied from 10-28 pairs, the accession Sivaganagai local have more leaflets with the range of 12-28 pairs. For all the accessions the leaflets are Oblong, sparely pilose on the margin. Leafy stipules are present in the size of 5-10mm. Long stipules were observed in Pant(6-12mm). The standard petal colour is yellow with dense stipes in the dorsal side. Sivagangai local and Pant had Dense Violet flecked. The pod was green in colour and non pubescent and the constriction is not prominent. The size of the pod ranged from 17.5-23 cm, Sivaganagi local had long pods with the length of 20-23 cm length and more number of seeds (34-36 seeds /pod). Plant height, base diameter, fresh weight and biomass (g per plant) and fresh and biomass yield (ton per hectare) of different dhaincha accessions were influenced by plant population densities, sowing time and fertilizer doses (Bisht, P. S., et al 1996).

Shahjalal, M. and Topps, J.H. 2000 found the positive correlation for total dry matter with seed width,1000 seed weight,shoot length and base diameter and the vigor index was positively correlated with seed length,shootlength,root length and germination and percentage and negatively correlated with base diameter.

Green Biomass yield and yield contributing distributors also varied due to the genetic makeup of dhaincha accessions (Evans, D.O. 1990). Yield contributing factors did not totally dependent upon the performance of individual plant but also on plant population per unit area.The plant height, leaflets per leaf,dryweight of plant have positive direct effect on biomass yield and used as selection criteria for the improvement of dhaincha biomass yield.((Sarwar,*et al*. 2015)

Salinity adversely affects the germination behaviour and seedling growing descriptors of dhainchagenotypes,the seed germination was relatively less affected by salt concentration compared to other growth descriptors. A significant variation among the dhaincha accessions in response to salt stress was also observed. Golamsarwar A.K.M *et al.* (2020). One ton of dhaincha biomass application to soil added approximately 33 kg N, 1 kg P, 14 kg K, 14 kg Ca, 16 kg Mg and 2 kg S (Bhuiyan*et al.)*

Plant height, base diameter, fresh weight and biomass (g per plant) and fresh and biomass yield (ton per hectare) of different dhaincha accessions were influenced by plant population densities, sowing time and fertilizer doses (Chun et al., 2007)

Nodulation studies

The greater number of root nodules were observed in Sivagangai local (52.4). pant variety showed the lowest value of (40.4) in Table 4. (Fig. 2)

Study of colony features and gram character

The colony characteristics and gram nature of the five chosen bacterial isolates (one from each plant sample) were recorded. The result is tabulated in Table 5.

Based on the study chosen isolates were gram-negative, rod-shaped bacteria which is the typical feature of the rhizobiaceae family as mentioned in the Bergey's manual of bacteriology. The colony characteristics of the four of the isolates were almost identical that indicates the strains might belong to the same species.*Rhizobium* colonies growth was observed after 24 hours of incubation among the four culture more rhizobium colonies were observed in Sivagangai local. The culture Vellore and Villupuram local shows less growth compared to Sivagangai local. The least colony growth was observed in pant variety.(Fig. 3)

CONCLUSION

The experimental research was conducted at ADAC& RI, Trichy, Tamil Nadu Agricultural University. Morphological characters *viz.,* plant height, number of root nodules per plant, plant population per m^2, fresh weight would be helpful for effective indication of *Sesbania* cultures *viz.,*Sivagangai local, Villupuram local, Vellore local & Pant variety in the field. Among the four cultures, green biomass yield of Sivagangai local was higher than those of other cultures.The number of Rhizobium colonies was higher in Sivagangai local as compared to villupuram local, Vellore local & Pant variety. This could further be utilized for their symbiotic effectiveness determination under field condition.

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Table 1:List of Daincha genotypes for characterization and nodulation

S.No.	Genotypes	Collected from
1	Sivagangai local	Sivagangaidistrict from Tamil Nadu
2	Villupuram local	Villupuram from Tamil Nadu
3	Vellore local	Vellore from Tamil Nadu
4	Pant	Pant

Table 2: Evaluation of Dhaincha accessions for Biometrical and traits

S.No.	Genotypes	PH I (cm)	PH II (cm)	NB/P	NP/P	NP/C	PL (cm)	NS/P	SW (g)	FW/P (g/plt)	GB (t/ha)	N.R N/P	PP /m^2
1	Sivagangai local	60	158	33	58	4-5	23.79	35-38	15.56	4.95	19.8	52.4	400
2	Villupuram local	66	137	23	47	4-5	20.12	32-35	17.41	4.35	15.2	43.2	350
3	Vellore local	65	130	25	38	4-5	20.45	30-35	16.73	4.10	13.0	48.8	320
4	Pant variety	61	122	25	27	3-5	19.52	30-32	15.04	3.90	11.3	40.4	285

PH I: Plant Height at 45 days, PH II-Plant height at maturity, NB/P-Number of Branches per plant,NP/P-Number of pods per plant, NP/C-Number of pods per cluster, PL-Pod length(cm), NS/P-Number of seeds per pod, SW- 1000 seeds weight, FW/P-Fresh weight per plant, GB/ha- Green biomass/ha, N.RN/P No. of root nodules/plant -PP/m^2- plant population/m^2

S.No.	Characteristics	Sivagangai local	Villupuram local	Vellore local	Pant
1	Plant anthocyanin coloration of hypocotyls	Absent	Absent	Absent	Absent
2.	Plant –branching pattern	Erect	Erect	Erect	Erect
3.	Time of flowering	45-50 days	48-55 days	42-48 days	45-50 days
4.	Plant growth habit	Determinate	Determinate	Determinate	Determinate
	Stem				
5.	Stem colour	Green with red long stripes	Green with brown strips	Green	Green
	Stem	Glabrous	Glabrous	Glabrous	Glabrous
6.	Stem pubescence	Sparsely pubacent when young	Sparsely pubacent when young	Sparsely pubacent when young	Sparsely pubacent when young
7	Leaf shapes	Paripinnate	Paripinnate	Paripinnate	Paripinnate
8	Leaf length	7-25 cm	6.5 to 24cm	7-23 cm	7.2-25.4 cm
9	No. of leaflets	12-28 pairs	10-25	12-27	12-25
10	Shape of leaflet	Oblong, sparely pilose on the margin	Oblong, sparely pilose on the margin	Oblong, sparely pilose on the margin	Oblong, sparely pilose on the margin
11	Stipules length	6-10mm	5-8mm	6-9mm	6-12mm
12	Leaf: Pubescence on lower surface of the leaf	Absent	Absent	Absent	Absent
	Flower: Colour of base of petal (Standard)	Yellow	Yellow	Yellow	Yellow
14	Flower: Pattern of streaks on petal (standard)	Dense Violet flecked	Dense red flecked	Dense dark Violet flecked	Dense Violet flecked
15	Pod : Colour	Green	Green	Green	Green
16	Pod: Pubescence	Absent	Absent	Absent	Absent
17	Pod: Waxiness	Absent	Absent	Absent	Absent
18	Pod: Surface stickiness	Absent	Absent	Absent	Absent
19	Pod: constriction	Not Prominent	Not Prominent	Not Prominent	Not Prominent
20	Pod size(cm)	20-23 cm	18-22cm	18-20 cm	17.5-20 cm
21	Pod: No. of seeds	34-36	30-35	28-32	25-30
22	Plant height	155-158 cm	135-148 cm	142-148 cm	148-150 cm
23	Seed colour	Brown	Brownish red	Greenish brown	Greenish brown

Table 3:Morphological descriptors studied in different accessions of D*haincha*germplasms

Table 4: Collection of plant root nodules from Dhainchafield.

Name of accessions	No. of root nodules per plant	No. of positive samples
Sivagangai local	5	3
Villupuram local	5	3
Vellore local	5	3
Pant	5	3

Table 5: Morphological characters of Rhizobium on different Dhaincha accessions

Name of accessions	Colony shape	Colony colour	Opacity	Margin	Elevation	Consistency	Gram character and shape of bacterium
Sivagangai local	Circular	White	Translucent	Entire	Raised	Mucoidal	Gram negative and rod shape
Villupuram local	Circular	White	Translucent	Entire	Raised	Mucoidal	Gram negative and rod shape
Vellore local	Circular	White	Translucent	Entire	Raised	Mucoidal	Gram negative and rod shape
Pant	circular	White	Translucent	Entire	Raised	Mucoidal	Gram negative and rod shape

Fig.1:Field view

Single plant

Fig 2.: Nodulation in Dhaincha genotypes

	
Sivagangai local	**Pant variety**
	
Vellore local	**Villupuram local**

Fig.3: Confirmatory test for Rhizobium cultures

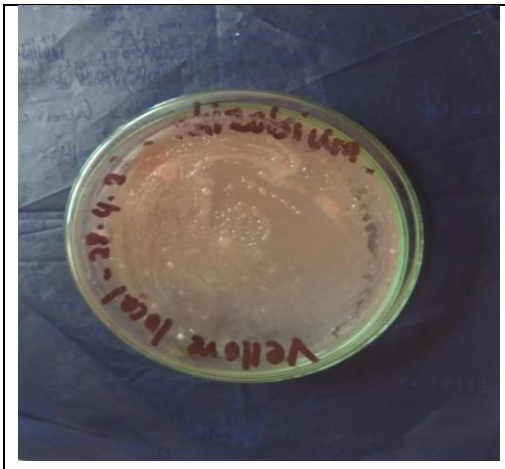
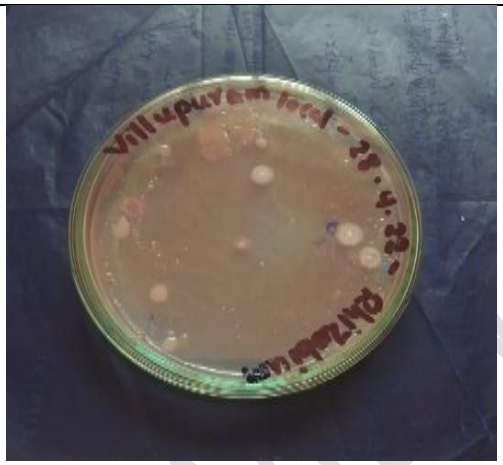
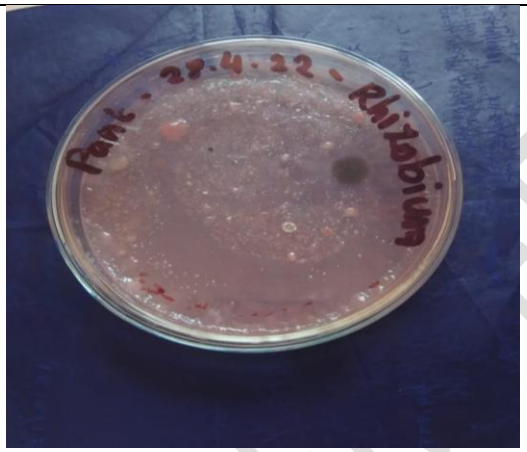
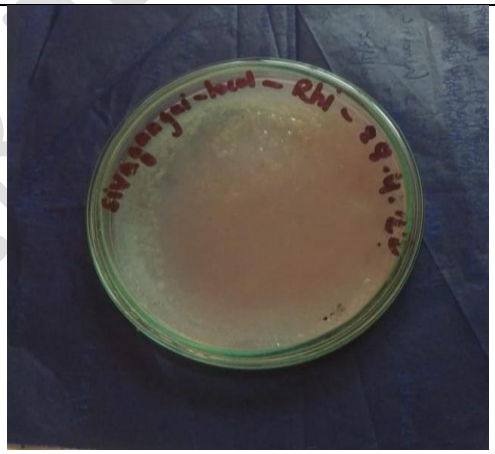
	
Sivagangai local	**Pant variety**
	
Vellore local	**Villupuram local**

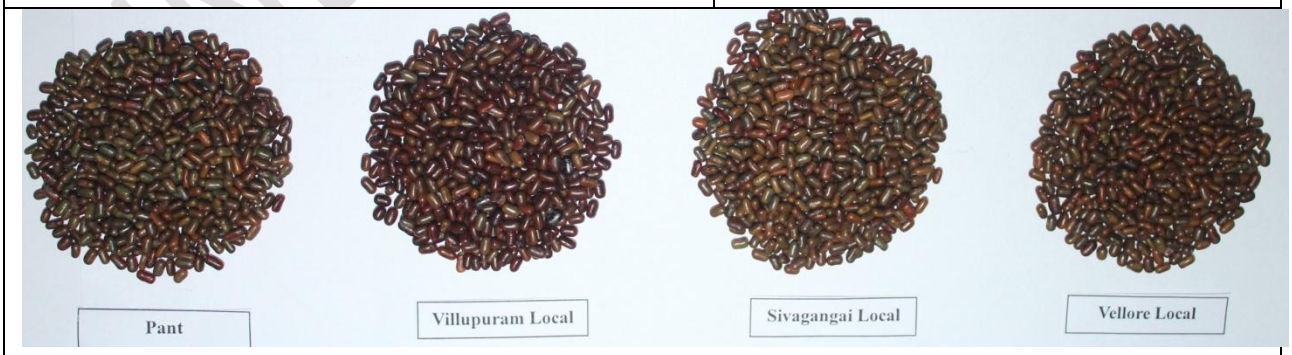
Fig 4: Morphological descriptions

Leaf and flower

Standard petal

Stem colour- reddish tinch

Pod colour- green



Seeds of Daincha accessions