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Diversity Assessment of Indian Sunnhemp (*Crotalaria juncea* L.) Accessions for Enhanced Biomass and Fibre Yield using Geographic Information System Approach

R.T. Maruthi, A. Anil Kumar, S.B. Choudhary[1], H.K. Sharma[2], J. Mitra

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ABSTRACT

Background: Sunnhemp, a rapid growing, high biomass yielding bast fibre crop has a tremendous potentiality in biofuels sector as a lignocellulosic substrate. In order to capitalize the new found area there is a need to identify high biomass and fibre yielding sunnhemp genotypes. The present study provides details of morphological diversity and geographical distribution pattern of Indian sunnhemp accessions.

Methods: A total of 42 germplasm accessions collected from ten different states were evaluated for fibre yield and attributing traits in April-June cropping season. Based on phenotypic data agglomerative hierarchical cluster analysis was performed. Geographical coordinates of germplasm collection site were utilized to derive the spatial genetic diversity pattern for green biomass yield and fibre yield.

Result: Phenotypic evaluation revealed significant genetic variability among the genotypes for biomass and fibre yield leading to identification of several promising accessions. Cluster analysis and PCA grouped the 42 sunnhemp accessions into three clusters. Cluster II and III are highly divergent harboring contrasting phenotypes. DIVA-GIS approach identified eastern Rajasthan, western Jharkhand and border area between Bihar and Jharkhand as sites of highest sunnhemp diversity.

Key words: *Crotalaria juncea* L., DIVA-GIS, Fibre yield, Green biomass, Multivariate analysis.

INTRODUCTION

Sunnhemp (*Crotalaria juncea* L.) is an annually renewable lignocellulosic non-wood bast fiber crop. It is one of the oldest fibre species from the Indian subcontinent being grown since 600 BC (Treadwell and Alligood, 2008). Traditionally the fibre is used for cot stringing, unsized twine, fishing nets, matting, sacking, marine cordage, coarse canvas, bags and ropes (Satya *et al.* 2016). Fibre pulp is ideal for making currency note paper, cigarette paper and high quality tissue paper (Chaudhary *et al*. 2015). Sunnhemp being a member of legume species has a great potential to be used as a soil improvement crop in the tropics. With its rapid growth habit, in 8-12 week the crop can produce biomass of 8900-13000 kg ha^{-1} and accumulates about 170-200 kg nitrogen per hectare. Roots have the ability to fix 50-60 kg ha^{-1} of atmospheric nitrogen through symbiotic association with rhizobium bacteria (Balkcom and Reeves, 2005). The crop has some additional benefits like it can produce adequate performance even in calcareous soils with high pH, reduce populations of sedentary parasitic nematodes such as root-knot (*Meloidogyne* spp.) nematodes (Wang *et al*. 2001) and effective in suppression of weeds. When it is grown as a cover crop, it will reduce soil erosion, conserve soil water, recycle plant nutrients and improve soil properties.

Apart from the conventional applications, sunnhemp has been evolved as an annually renewable non-food substrate for lignocellulosic biofuel production. Sunnhemp crop can be easily grown during fallow periods between major cash crops with minimum inputs, which helps in sustainable use of limited land and water resources and solves the problem of "food vs. fuel" debate for biofuel production. Sunnhemp a fast growing, high biomass yielding crop can produce 10.7 Mg ha^{-1} of biomass with an energy content of 19.0 Mg ha^{-1} in 90 days, which resulted in an energy yield of 204 GJ ha^{-1} (Cantrell *et al*. 2010). Sunnhemp fibre is rich in cellulose, containing 75.6% cellulose, 10.05% hemicellulose, 10.32% lignin, with high crystallinity (80.17%) and degree of polymerization (650). Using "microwave-assisted ionic liquid-mediated rapid catalytic conversion" method lignocellulosic

Crop Improvement Division, ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata-700 121, West Bengal, India.
[1]ICAR-NBPGR Regional Station, Ranchi-834 010, Jharkhand, India.
[2]ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur-321 303, Rajasthan, India.

Corresponding Author: R.T. Maruthi, Crop Improvement Division, ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata-700 121, West Bengal, India.
Email: maru7806@gmail.com

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sunnhemp fibres can be converted into biofuels in just 46 min. Further, it was found that 1 kg of sunnhemp fibres containing 756 g of cellulose yielding 595 g of glucose and 203 g of hydroxymethyl furfural (furanic biofuel precursor), further fermentation of glucose produces 75.6% bioethanol yield (Paul and Chakraborty, 2018). Biofuels produced from non-food lignocellulosic feedstocks like sunnhemp have the potential to replace a significant percentage of fossil fuels (Sadhukhan and Sarkar, 2015).

In spite of having enormous industrial applications, area under the crop reduced drastically (Chaudhary *et al*. 2015). In most parts of the country, sunnhemp is cultivated primarily as a green manure crop not as a fibre crop. One of the reason being the unavailability of consistent high yielding varieties. Hence, there is a need to redesign the breeding programs by using newer and diverse germplasm to improve the yield and quality of sunnhemp fibre. The knowledge of genetic variability is completely necessary for introducing the breeding programs. However, limited information on genetic diversity of Indian Crotalaria species is available (Pandey *et al*. 2010; Subramaniam *et al*. 2013; Satya *et al*. 2016; Maruthi *et al*. 2020; Desai *et al*. 2021). In this endeavor, the present investigation was undertaken to understand the existing variability of Indian sunnhemp accessions for enhanced biomass and fibre yielding potentiality and to identify the genotypes for population improvement program. Further, the geographical areas with high variability for these traits may be highlighted using DIVA-GIS approach which is useful for planning future germplasm exploration and conservation activities.

MATERIALS AND METHODS

Plant material and field evaluation

The plant material used in present study comprised of 42 germplasm accessions assembled from ten different states of India (Fig 1). The germplasm was evaluated in randomized complete block design with three replications and involving two check varieties K 12 Yellow and K 12 Black. The field experiment was conducted at ICAR-Central Research Institute for Jute and Allied Fibres, Kolkata, India (22°45’N, 88°26’E; 9 msl) in April-June cropping season. Each accession was sown in two rows of 4 m row length with 30 x 5-7 cm spacing. No fertilizers were applied during the crop growth and development with an intention to determine the yield potentiality of the crop under low-input conditions (Nkoana *et al*. 2019).

Phenotyping of morphological traits and statistical analysis

At 90 days after sowing, five random plants were harvested from the middle of rows of each accession and observations were recorded on plant height (PHT; cm), basal stem diameter (BD; mm), green biomass yield (GBY; g/plant). The harvested green stem was retted in a water tank for 48 hrs to extract the bast fibre, which was air-dried prior to measuring wood yield (WY; g/plant) and fibre yield (FY; g/plant). Mean data from five plants was used for further data analysis.

The descriptive statistics for morphological traits were calculated using statistical tools for agricultural research version 2.0.1 (STAR, 2014). Genetic parameters were estimated using the formulae suggested by Singh and Chaudhary (1977). Manhattan distance was calculated from pair wise comparisons of the genotypes for all the traits and the distance matrix were then subjected to agglomerative hierarchical cluster analysis to produce a dendrogram. The data was than subjected to principal component analysis (PCA), to ascertain the traits that effectively differentiated the 42 accessions. DIVA-GIS version 7.5 (www.DIVA-GIS.org) was used for geographical mapping of genetic diversity and to assess spatial genetic diversity pattern for specific traits *viz*., green biomass yield and fibre yield.

RESULTS AND DISCUSSION

Morphological variability

The genotypes revealed wide range of phenotypic expression for all the traits under study (Table 1). The mean plant height was 312.12 cm, but it ranged from 268.50 cm for accession SIN-24 to 349.92 cm for accession SIN-28. Four accessions *viz*., SIN-28 followed by SIN-22, SIN-11 and SIN-06 were found taller than check variety K 12 black (334.00 cm) (Table 2). Average basal stem diameter was 14.03 mm while range was 10.68-17.44 mm for SIN-35 and SIN-06. Since bast fiber occurs in phloem or bark surrounding the plant stem, fibre yield improvement can be achieved primarily by selecting genotype with better plant height and basal stem diameter (Chen *et al*. 1990; Sawarkar *et al*. 2014).

Fig 1: DIVA-GIS map showing collection sites of *C. juncea* accessions.

Table 1: Statistical parameters of genetic variability in *C. juncea*.

Traits	Range	Mean±SD	CV(%)	Mean square genotype (DF=41)	*F*
PH	283.81-349.92	312.12±12.29	4.64	453.0918	2.16
BD	10.68-17.44	14.03±1.36	11.5	5.5587	2.14
GY	181.07-456.40	276.98±55.35	5.32	9189.837	42.27
FY	3.21-11.95	6.68±1.72	18.96	8.8266	5.51
SW	30.89-75.57	48.02±10.50	13.84	330.4389	7.48

PH: Plant height (cm), BD: Basal diameter (mm), GY: Green biomass yield (g/plant), FY: Fibre yield (g/plant), SW: Stick yield (g/plant).

Table 2: Genotypes identified based on their superior performance for morphological traits.

Traits	Performance of K 12 Black*	No. of genotypes superior to K 12 Black	Best performing genotype
PH	334.00	4	SIN-28 (349.92)
BD	14.11	21	SIN-06 (17.44)
GY	296.97	12	SIN-07 (456.40)
FY	8.81	3	SIN-07 (11.95)
SW	62.28	3	SIN-07 (75.57)

* Check variety.

Green biomass yield, an important yield character, varied extensively from 181.07 g/plant to 456.40 g/plant with an average of 276.98 g/plant. Similarly, wood yield per plant also revealed significant variation with a range of 30.89-75.57 g. Accession with high green biomass yield and average wood yield are more desirable since this trait combination ensures high fibre recovery percentage, which eventually leads to higher fibre yield (Kumar *et al*. 2018). For fibre yield, the range was 3.21-11.95 g/plant with accessions SIN-07, SIN-11 and SIN-15 having 11.95 g, 10.58 g and 9.99 g fibre yield per plant, respectively, which is significantly higher than check variety K 12 black (8.81 g/plant). The accessions SIN-06, SIN-07, SIN-11, SIN-15 and SIN-22 that recorded higher values for majority of the yield contributing traits were collected from different districts of Rajasthan.

Cluster analysis

Cluster analysis performed using Manhattan distance matrix and Average linkage method of minimum variance grouped the accessions into three major clusters (Fig 2). Accession SIN-10 and SIN-12 from Rajasthan, SIN-27 and SIN-35 from Jharkhand and SIN-24 from Kerala formed part of cluster III, distinctly separated from the rest of the accessions. SIN-06, SIN-07 and SIN-11 another accessions from Rajasthan featured in cluster II, along with SIN-02 an accession from Kerala. The rest of the accessions did not show any specific pattern based on geographic origin and were grouped in cluster I. Satya *et al*. (2016) reported moderate to narrow genetic variability with fibre type India sunnhemp cultivars and breeding lines forming a single group. High genetic distance was observed between cluster II and III indicating the accessions from these clusters are distinct from each other. Inter-mating of these genotypes is expected to generate high population variability. Hence, it suggests use of these germplasm in population improvement program to break the yield barrier in sunnhemp.

Table 3: Principal Component loadings for fibre yield and associated traits in *C. juncea*.

Variables	PC1	PC2	PC3
Plant height (cm)	-0.38	-0.76	0.44
Basal diameter (mm)	-0.46	0.11	-0.56
Green biomass yield (g/plant)	-0.49	0.14	-0.17
Fibre yield (g/plant)	-0.40	0.60	0.66
Stick weight (g/plant)	-0.49	-0.15	-0.19
Eigen values	3.78	0.62	0.36
Proportion of variance (%)	75.51	12.36	7.18
Cumulative variance (%)	75.51	87.87	95.05

Principal component analysis

PCA of forty-two Indian sunnhemp accessions based on correlation matrix revealed three principal components that together accounted for 95 per cent of total multivariate variation (Table 3). In the first PC (75.5% of total variation), the traits with significant contribution were basal stem diameter, green biomass yield and wood yield. The second PC (12.4% of the total variation) accounted for variation mainly with respect to plant height and fibre yield. Also an attempt has been made to plot the 42 accessions in a plane defined by the two principal components (Fig 3). Only few accessions with same geographical origin were grouped together while majority of the population together formed a big cluster in the center of the plane irrespective of their eco-geographical zones as also revealed by cluster analysis. Both cluster analysis and PCA analysis produced comparable results, a high correspondence between these two results is indicative of robustness of the present classification. Satya *et al*. (2016) while studying species diversity in *C. juncea* using SCoT markers, obtained similar kind of congruence. The cluster II formed a distinct group in the top right of the plane; these accessions were taller with high fresh biomass and dry fibre yielders among the

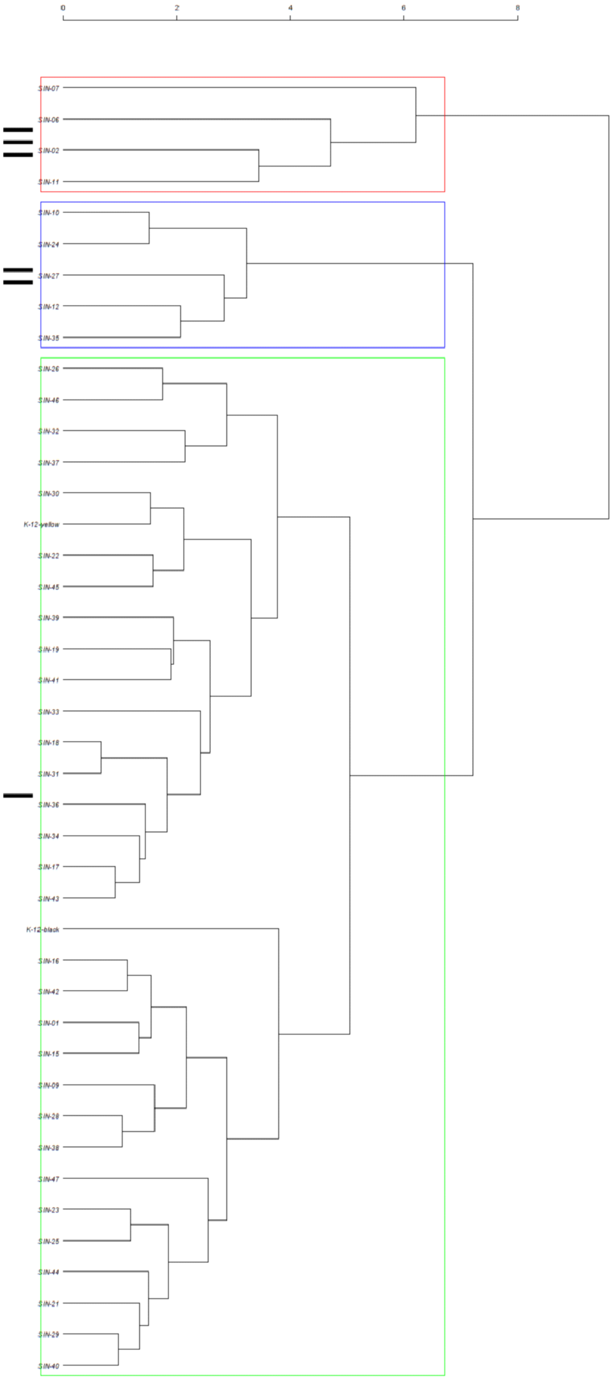

Fig 2: Dendrogram using Agglomerative clustring method.

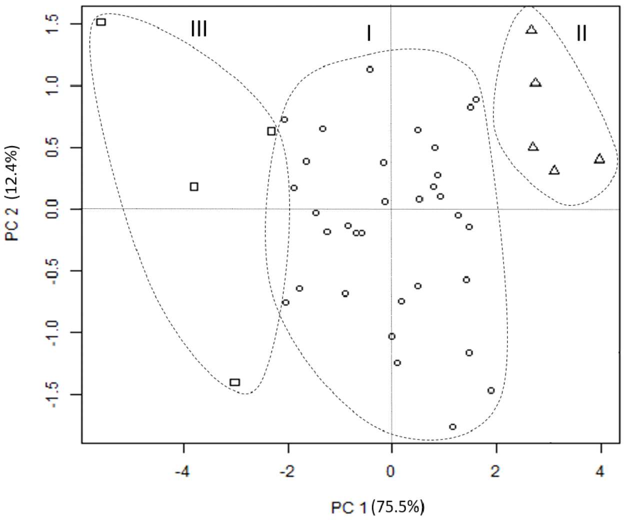

Fig 3: Biplot of forty-two Indian sunnhemp accessions along the first and second principal component vectors based on fibre yield and associated traits. Clusters are indicated in Roman numericals.

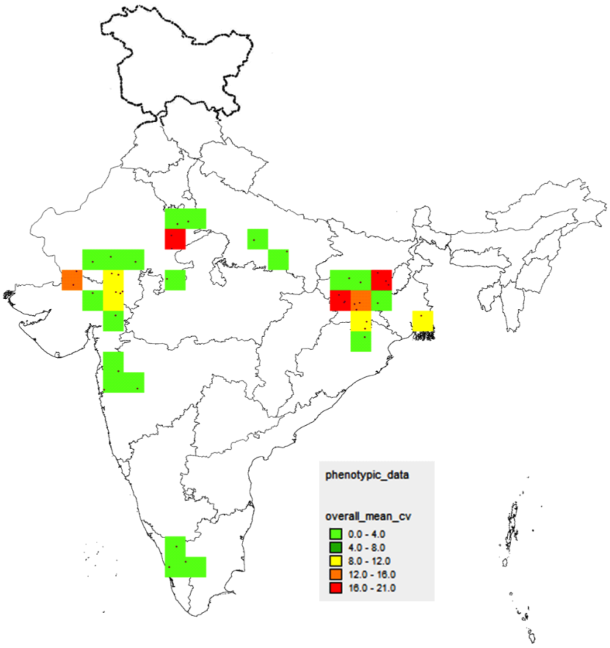

Fig 4: Coefficient of Variation (%) of *C. juncea* accessions using DIVA-GIS.

genotypes. Cluster III composed of four accessions scattered in the left of the plot; these accessions were low yielders with shorter plant height compare to other groups. Cluster I, the accessions distributed equally on upper and lower half of the plot in the middle, comprised of thirty-three accessions, which recorded intermediated agronomic performance comparable to the best accessions as well as checks.

DIVA-GIS approach

Coefficient of variation (CV) is a simplest measure to analyze diversity of a given population. CV of all the five morphological traits were combined to generate a single grid map showing average CV of the five layers (Fig 4). The map will help to visualize the spatial diversity of sunnhemp accessions. The sites with the highest sunnhemp diversity are located in eastern Rajasthan, western Jharkhand and

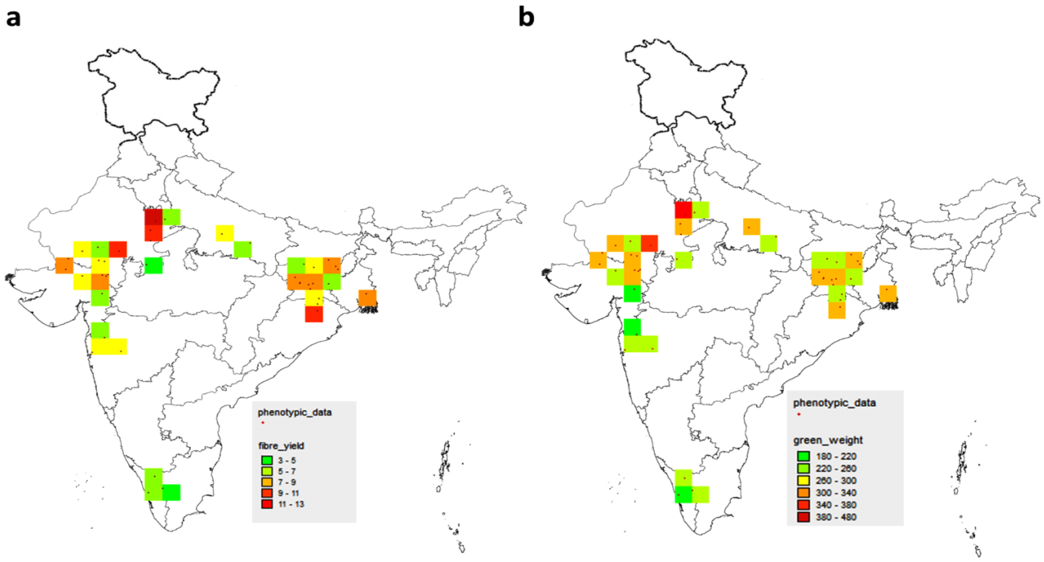

Fig 5: DIVA-GIS grid maps showing diversity index for (**a**) green biomass yield and (**b**) fibre yield/plant in sunnhemp germplasm.

in the border area between Bihar and Jharkhand. These geographical areas are known to harbor maximum genetic variability for *C. juncea* (Pandey *et al*. 2010). Further, grid map for specific traits *viz.*, green biomass yield and fibre yield (Fig 5) was generated and locations with highest phenotypic value for the trait was identified. Sunnhemp accessions with high green biomass yield were found mainly in eastern and southern districts of Rajasthan (Pali, Alwar, Dausa and Sawai Madhopur) and parts of northern Orissa region. Whereas, only accessions from eastern and southern districts of Rajasthan were turned out to be high fibre yielders, which means these accessions has got good fibre recovery percentage. Similar studies using GIS approach in assessing the genetic diversity and in mapping areas of high diversity have also been reported in different crop species (Parthasarathy *et al*. 2006; Sunil *et al*. 2009; Raina *et al*. 2015). GIS mapping of phenotypic data combined with passport data aided in spatial analysis of diversity pattern in sunnhemp.

The present investigation revealed significant variability among the Indian sunnhemp accessions. The genetically diverse and agronomically superior accessions identified through this present study could be potentially utilized as parents for introgression of favorable traits and develop broad genetic based population to derive varieties with high biomass and fibre yielding ability.

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