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DIVA-GIS based insight into geographical distribution and diversity spectrum of Indian sunnhemp (*Crotalaria juncea* L.) accessions suitable for diversified applications

R.T. Maruthi[1], A.A. Kumar[1], S.B. Choudhary[2], H.K. Sharma[3], Jiban Mitra[1]

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ABSTRACT

Commercial prospects of sunnhemp inspired present study to understand geographical distribution pattern(s) and to scale agro-morphological diversity spectrum of forty-four sunnhemp accessions naturalized across diverse habitats of India. Field experiment revealed broad spectrum diversity for all the 11 agro-morphological traits. Wider range of plant height (110.50 to 173.17 cm), number of pods per plant (35.33 to 143.00), seeds per pod (6.33-15.17g) and seed yield per plant (8.27-29.43g) highlighted the adequacy of present genetic resources to improve sunnhemp for diversified applications. Principal component analysis of the agro-morphological characters identified the first PC with 1109.6 eigen value explaining 61.70% of total variation followed by PC-II (22.9%) and PC-III (11.1%). In PC-I significant contribution was made by traits like NLP, NPP and PH. Agglomerative hierarchical cluster analysis grouped all accessions into four distinct seed producing clusters irrespective of their origin. Cluster wise mean values suggested that cluster-II is the best with outstanding trait values for majority of traits. DIVA-GIS based analysis identified accessions from Rajasthan, Western Gujarat and Jharkhand with high diversity index for number of leaves/plant. But, accessions from North West Jharkhand and Maharashtra with highest diversity index for seed yield/plant.

Key words: Agro-morphological traits, Cluster analysis, Cover crop, *Crotolaria juncea* L., DIVA-GIS.

INTRODUCTION

Sunnhemp (*Crotalaria juncea* L.) is one of the earliest (400 B.C.) domesticated crops valued for its multipurpose applications. In India, being the most distinctly named fibre crop (Sarkar *et al*., 2015), this species earned several locale names including Indian hemp, Madras hemp, brown hemp, Tropic sun *etc*. Stem bark of the plant produces phloem fibre commercially valued for durability, lighter colour and resistant to moisture, mildew, and micro-organisms (Maiti *et al*., 2010). The fibre found even better than jute (*Corchorus olitorius*) for durability and scored better fibre length (3.79 vs. 2.62 mm) and width (24.3 vs. 19.7 µm) over kenaf (*Hibiscus cannabinus*) (Cunningham *et al*., 1978). As a result its commercial prospects in traditional industries like preparation of cordage, fishing nets, tatpatti, ropes, twines, canvas and floor mats have not only consolidated over the years but also expanded to new horizons like manufacturing of pulp, paper, newsprint and specialty papers.

In addition, the species traditionally valued as a green manure crop and often grown in rotation with several different crop species across tropical and subtropical regions (Linares *et al*., 2007; Tripathi *et al*., 2013). With rapidly establishing canopy the crop found effective competitor of common field weeds besides producing 5.9 to 7.6 t ha^{-1} of biomass within 12 to 14 weeks and fix 120 to 144 kg ha^{-1} nitrogen over 9 to14 weeks (Balkcom and Reeves, 2005; Bhardwaj *et al*., 2005). Further, the species is an outstanding soil organic matter augmenter and carbon sequester. Its potentiality to suppress plant pathogenic nematodes like root knot nematode populations, encourage its use as an alternative to nematicides (Pradhan *et al.,* 2001; Wang *et al*., 2001).

Despite such a broad spectrum commercial prospects and emerging opportunities, area under sunnhemp cultivation is stagnated. In 2014-15, the crop occupied an area of 11.6 thousand hectares that produces 46.8 thousand bales of fibre (1 bale = 180 kg) with a national productivity of 7.3 q ha^{-1} (DAC&FW, 2017). With the growing carbon footprints of developmental initiatives in the post industrialization era and input intensive farming practices during post green revolution, interests in the crop further renewed. However, area under crop and its productivity could

[1]Crop Improvement Division, ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata-700 120, West Bengal, India.
[2]ICAR-NBPGR, Regional Station, Ranchi-834 010, Jharkhand, India.
[3]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-700 120, West Bengal, India.
Email: maru7806@gmail.com

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not match with the growing global demand that seriously compromised its commercial prospects. Although, some cultivar improvement programs are being undertaken at ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata, India, still more serious efforts are required for diversified applications.

Measuring diversity spectrum of important agro-morphological traits and their geographical distribution is of paramount importance in this endeavor. The ever growing gap between demand and supply of sunnhemp seeds are another challenge warranting serious scientific intervention. The limited seed supply is primarily attributed to photoperiod sensitivity of the crop as it flowers only under short day conditions (White and Haun, 1965). Further, the crop reported to be a self-incompatible and cross-pollination is the preferred mode of reproduction, only large bees from the *Xylocopa* or *Megachile* genera are capable of effecting pollination (Cho *et al*., 2016; Hall and Avila, 2016; Thimmaiah *et al*., 2018). Therefore, seed yield in sunnhemp is mainly depends on two things; availability of the bee population in the locality (Free, 1993) and number of flowers per plant. The first component is largely depends on environment, while the second factor is determined by the genetic potential of the genotype where breeding interventions can be made (Abdul-baki *et al*., 2001; Cho *et al*., 2016). Genotypes which can produce more number of branches and bear more number of flowers have a favorable effect on seed yield. In addition, the plant type with more branches will have enhanced weed suppressing ability and high biomass yield.

In this backdrop, present work was undertaken to scale agro-morphological diversity spectrum of selected sunnhemp genetic resources from India and to highlight their geographical distribution pattern(s). Findings of the present work will prove enabling baseline information for future breeding programs targeting at development of cultivar(s) suitable for diversified applications.

MATERIALS AND METHODS

Plant material and design of experiment

The experimental material comprised forty-four accessions of sunnhemp (*C. juncea* L.) collected from different agro ecological regions, spreading across the states of India like Rajasthan (14), Jharkhand (10), India (4), Bihar (3), West Bengal (3), Gujrat (3), Maharashtra (3), Tamil Nadu (2), Kerala (1) and Odisha (1) (Table 1). Two released cultivars (K 12 Yellow and K 12 Black) of sunnhemp were included in the study as checks. The map of collection sites is provided in Fig 1. All the accessions were grown in a completely randomized block design with three replications at the experimental research farm of the ICAR-Central Research Institute for Jute and Allied Fibres (ICAR-CRIJAF), Kolkata, India. The experimental farm is located at 22°45′N latitude and longitude of 88°26′E with 9 m above mean sea level. Each accession was represented by a single row of 4 m length planted at 50 cm apart and plant to plant spacing of 5-7 cm. Standard package of practices were adopted to raise good crop free of any abiotic and biotic stresses.

Observation on agro-morphological traits

Data on vegetative phenotypic characteristics like plant height (PH; cm), number of primary branches/plant (NPBP), number of secondary branches/plant (NSBP), number of internodes/plant (NIP), internodal length (IL; cm) were recorded on five randomly selected plants in each replication at 45 days after sowing (DAS). Reproductive phenotypic characters like days from seeding to date of 50% open ûower (DFF), number of pods/plant (NPP), number of seeds/pod (NSP), seed yield/plant (SYP; g/plant) and hundred seed weight (HSW; g) were recorded at the seed maturity. Data on pod characters were recorded from the same five sample plants used to assess vegetative traits.

Statistical analysis

Statistical analysis of the collected data was done by using statistical tools for agricultural research version 2.0.1 (STAR, 2014). Data on 11 agro-morphological traits were examined for analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) to identify the number of homogenous groups. The genotypic and phenotypic components of variance, coefficients of variability, broad sense heritability and genetic advance were assessed by applying the formulae recommended by Singh and Chaudhary (1977). Further, morphological data were examined using Euclidian distance coefficient. The resultant distance matrix was used to produce a dendrogram for which the agglomerative hierarchical cluster analysis (AHC) and the ward's clustering method was employed for depicting the genetic relationships (Everitt *et al*., 2001). Data were then subjected to principal component analysis (PCA) using the same statistical program. The eigen vectors and eigen values were determined in the principal component analysis. To know the spatial distribution and assessment of variability DIVA-GIS version 7.5 (www.DIVAGIS.org) was used. Maps on the genetic diversity pattern of selected traits *viz*., NLP and SYP were generated in coordination with geographical coordinates (latitude and longitude) of the collection site.

RESULTS AND DISCUSSION

Morphological variability

In the present study, a wider spectrum of diversity for all the 11 agro-morphological traits revealed as CV ranged from 1.39 to 19.84 (Table 2). Number of the accessions started flowering later than the commercial cultivars (checks) and the mean days to 50% flowering recorded around 45.61 (±4.68). Delayed onset of flowering extended vegetative growth period that led to improved biomass accumulation. The phenomenon is of paramount importance for high biomass breeding program (White and Haun, 1965; Cho *et al*., 2016). However,

three accessions namely SIN-30, SIN-01 and SIN-26 recorded early flowering, with 50% flowering at 33 days after sowing (Table 3). These accessions are of particular interests for understanding flowering behavior and associated mechanism in sunnhemp. Plants with taller growth habit together with higher number of leaves as well as branches favor higher biomass accumulation (Cho *et al*., 2016). These component traits attract breeders aiming at improving sunnhemp as a cover crop. Further, these accessions recorded high degree of variability for plant height that ranged from 110.50 (SIN-30) to 173.17 cm (SIN-09) with a mean value of 142.15±14.46 cm. One of the accession namely SIN-

Table 1: Place of collection of forty-four accessions of sunnhemp (*Crotalaria juncea*).

Code	Collector no., if any	Place of collection	Latitude	Longitude
SIN-01	NATP/BHR/MG/025	Bihar	23.4	84.7
SIN-02	NATP/MP/025	Orissa	21.71	85.7
SIN-06	NYM-2845	Rajasthan	26.04	76.35
SIN-07	NYM-2852	Rajasthan	27.24	76.62
SIN-09	NYM-2858	Rajasthan	27.35	77.14
SIN-10	YBM-51	Rajasthan	25.38	74.58
SIN-11	YBM-65	Rajasthan	24.56	76.11
SIN-12	YBM-75	Rajasthan	25.26	74.48
SIN-15	KBA-198	Rajasthan	24.93	71.73
SIN-16	KBA-208	Rajasthan	23.91	73.64
SIN-17	KBA-211	Rajasthan	23.86	73.85
SIN-18	KBA-213	Rajasthan	23.94	73.96
SIN-19	KBA-214	Rajasthan	24.76	73.76
SIN-21	KBA-218	Rajasthan	24.82	73.43
SIN-22	KBA-220	Rajasthan	25.64	73.38
SIN-23	KBA-225	Rajasthan	25.38	72.51
SIN-24	NM-2/15	Kerala	10.48	76.21
SIN-25	JBT-30/22	Bihar	24.85	86.33
SIN-26	JBT-30/33	Bihar	24.59	84.93
SIN-27	JBT-30/62	Jharkhand	23.78	84.45
SIN-28	JBT-30/78	Jharkhand	23.05	85.29
SIN-29	SKJ-1/51	Jharkhand	24.38	85.49
SIN-30	YMG-310	Gujrat	24.09	72.42
SIN-31	YMG-321	Gujrat	23.36	73.08
SIN-32	YMG-327	Gujrat	23.2	73.57
SIN-33	NLA-3/26	Tamil Nadu	10.74	77.02
SIN-34	NA-3/48	Tamil Nadu	11.48	76.57
SIN-35	JBT-33/106	Jharkhand	24.45	86.72
SIN-36	JBT-36/34	West Bengal	23.37	86.32
SIN-37	JBT-36/118	Jharkhand	23.38	85.45
SIN-37-1	-	Jharkhand	23.38	85.45
SIN-38	JBT-36/124	Jharkhand	23.33	85.2
SIN-39	JBT-27/57	Jharkhand	23.44	84.72
SIN-40	JBT-38/51	Jharkhand	22.47	85.79
SIN-40-1	-	Jharkhand	22.47	85.79
SIN-41	JBT-38/133	Jharkhand	22.13	85.7
SIN-42	DHS-5	India*		
SIN-43	Bidhan Shau	India*		
SIN-44	AMDJ-4/28	Maharashtra	19.21	74.68
SIN-45	AMDJ-4/45	Maharashtra	19.15	73.06
SIN-46	AMDJ-4/52	Maharashtra	20.05	73.76
SIN-47	JBT-41/89	Jharkhand	24.27	86.84
K-12-Yellow	-	India*		
K-12-Black	-	India*		

*Place of collection not known.

29 produced the highest number of leaves per plant (162.33), while the average number of leaves per plant was 104.74±29.56 (range: 32.33-162.33). Similarly, accessions SIN-38 and SIN-41 were selected for superior branching habit.

Scanty availability and unreliable supplies of quality seeds are a serious limiting factor compromising the prospects of sunnhemp as a cover crop. Significant variability observed for seed yield attributing traits in the present study. Number of pods per plant ranged from 35.33 (SIN-07) to 143.00 (SIN-47) with an average of 75.59±24.24. Interestingly number of seeds per pod recorded high CV (11.52) and also showed wide variability (range: 6.33-15.17). Thus, high coefficient of variation and range indicates that phenotypic selection is adequate to bring desirable improvement in these characters (Boghara *et al*., 2016). Seed yield per plant varied from 8.27-29.43 g with mean value 16.36±5.17 g per plant. Particularly, accession, SIN-28 recorded the highest seed yield per plant (29.43 g/plant) followed by SIN-47 (23.30 g/plant), SIN-44 (27.03 g/plant) and SIN-34 (25.23 g/plant).

ANOVA and analysis of phenotypic and genotypic variances

Analysis of variance discovered sizable variations ($P < 0.01$) among the genotypes for all the 11 traits indicating broad spectrum diversity in the experimental material (Table 2). Duncan's multiple range test identified 11 (for internode length) to 24 (for plant height) significantly different homogeneous groups in the sunnhemp germplasm accessions, with an average of 18.09 groups. The variance was moderate to high for nearly all the traits examined as phenotypic coefficient of variation (PCV) which ranged from 10.34 to 46.15% and genotypic coefficient of variation (GCV) ranged from 10.08 to 41.84% (Table 2). Although higher value of PCV over GCV for all the eleven characters apparently reflects a fair genotype x environmental interactions in the species, narrow difference between PCV and GCV suggested limited influence of environment on expression of these traits. Therefore, selection for enhancing such traits will be rewarding. The higher estimates and close agreement of PCV with GCV for number of secondary branches, number of primary branches, seed yield per plant and number of pods per plant are encouraging to breed for biomass improvement as well as seed yield in the species.

In general, broadsense heritability ($\hat{h}^2_{BS}$) was higher (> 70%) for all the traits except internode length (47%). Precisely, 100 seed weight recorded the highest broad sense heritability (99%) followed by number of leaves per plant (98%), days to fifty percent flowering (97%) and number of pods per plant (97%). The phenomenon underscored the high degree of transmissibility of these traits from one generation to another with least environmental fluctuations. Percentages of genetic advance (GA) ranged from 16.94 to 78.13% (Table 2).

Table 2: Estimates of mean performance, mean square and genetic variables for 11 characters in 44 sunnhemp accessions.

Characters	Mean Performance				Mean Square	Homogeneous groups[#]	Genetic Parameters					
	Mean±SD	Range	SEm	CV (%)	Genotype (DF=43)		σ^2g	σ^2p	GCV	PCV	H_2 (%)	GA(%) Mean
DFF	45.61±4.68	33.00-53.67	0.51	1.92	65.82**	22	21.68	22.45	10.21	10.39	97	20.67
PH	142.15±14.46	110.50-173.17	1.86	2.27	626.88**	24	205.50	215.89	10.08	10.34	95	20.27
NLP	104.74±29.56	32.33-162.33	2.47	4.09	2621.94**	22	867.86	886.22	28.13	28.42	98	57.34
NPBP	7.86±2.67	3.33-15.17	0.90	19.84	21.41**	13	6.33	8.76	32.00	37.65	72	56.02
NSBP	9.63±4.17	1.83-17.50	1.08	19.48	52.25**	18	16.24	19.76	41.84	46.15	82	78.13
NIP	34.44±5.51	21.67-46.00	1.95	9.81	91.12**	14	26.57	37.98	14.97	17.89	70	25.79
IL	2.52±0.36	1.85-3.33	0.19	12.80	0.38**	11	0.09	0.20	12.01	17.56	47	16.94
NPP	75.59±24.24	35.33-143.00	2.39	5.46	1768.59**	23	582.05	599.06	31.91	32.38	97	64.80
NSP	9.88±1.97	6.33-15.17	0.66	11.52	11.60**	15	3.43	4.73	18.76	22.02	73	32.93
SYP	16.36±5.17	8.27-29.43	1.05	11.07	80.26**	16	25.66	28.94	30.96	32.88	89	60.05
HSW	3.64±0.54	2.74-4.89	0.03	1.39	0.86**	21	0.29	0.29	14.70	14.77	99	30.16

*, ** significant at 5% and 1% level of significance.

[#]Number of significantly different homogeneous groups produced by Duncan's multiple range test.

SD: Standard deviation; SEm: Standard error of mean; CV: Coefficient of variation; σ^2g: genotypic variance, σ^2p: phenotypic variance, GCV: genotypic coefficient of variation, DF: Degree of freedom; PCV: phenotypic coefficient of variation, H_2: Broad sense heritability and GA: genetic advance.

Table 3: Accessions identified for superior agro-morphological traits.

Characters	Best performing accession
DFF	Early-SIN-30, SIN-01, SIN-26 (33.44); Late-SIN-18, SIN-22, SIN-27 (53.67)
PH	SIN-09 (173.17 cm)
NLP	SIN-29 (162.33)
NPBP	SIN-38 (15.17)
NSBP	SIN-38, SIN-41 (17.50)
NIP	SIN-27, SIN-28, SIN-23 (46.00)
IL	SIN-01 (3.33 cm)
NPP	SIN-47 (143.00)
NSP	SIN-44 (15.17)
SYP	SIN-28 (29.43 g/plant)
HSW	SIN-07 (4.89 g)

Table 4: Eigen vectors, eigen values and proportion of variance of the first five principal components for different traits of sunnhemp accessions.

Variables	Eigen vectors				
	PC-I	PC-II	PC-III	PC-IV	PC-V
DFF	-0.065	-0.024	0.047	-0.466	-0.585
PH	-0.113	0.226	0.939	0.214	-0.009
NLP	-0.818	-0.563	0.026	0.072	-0.014
NPBP	-0.047	-0.019	-0.025	-0.040	0.087
NSBP	-0.067	-0.016	-0.020	-0.033	0.686
NIP	-0.072	0.046	0.203	-0.850	0.304
IL	0.006	0.001	0.000	0.030	0.001
NPP	-0.541	0.784	-0.268	0.033	0.013
NSP	-0.031	0.004	-0.006	0.017	-0.137
SYP	-0.093	0.117	0.028	-0.062	-0.258
HSW	0.008	0.000	0.009	0.019	0.045
Eigen values	1109.604	411.268	199.402	27.249	18.775
Proportion of variance (%)	61.70	22.90	11.10	1.50	1.00
Cumulative variance (%)	61.70	84.50	95.60	97.10	98.20

Traits like number of leaves per plant, number of primary branches per plant, number of secondary branches per plant, number of pods per plant and seed yield per plant recorded high heritability and genetic advance that adds confidence in improving these traits through cost effective, simple methods of phenotypic selection (Yamada, 2001).

Principal component analysis

Principal component analysis (PCA) of the eleven agro-morphological characters, based on the correlation matrix revealed that the first PC had 1109.6 variance (eigen value) which explained 61.70% of total variation (Table 4), followed by PC-II (22.9%) and PC-III (11.1%). Cumulatively, first five principal components accounted for 98.2% of the total variation. In PC-I significant contribution was made by NLP followed by NPP and PH.

Cluster analysis and DIVA-GIS

Agglomerative hierarchical cluster analysis using 11 agro-morphological traits grouped 44 accessions of sunnhemp into well-defined phenotypes with four distinct seed producing clusters (Fig 2). Cluster-I and cluster-III harbored 11, 13 accessions, respectively producing medium seed yield. Cluster-II consisted of 13 high seed producing accessions, while cluster-I consisted of 3 low seed producing accessions. All major clusters consisted of a mixture of genotypes from different places of collection. This suggested that the geographical diversity and genetic diversity are not related in the studied sunnhemp gemrplasm lines. Cluster wise mean values of 11 traits (Table 5) suggested that cluster-II is the best with outstanding trait values for the majority of vegetative and reproductive traits like PH, NPBP, PSBP, NPP, NSP and SYP. It is closely followed by cluster-I which had highest mean value for NLP and average performance of other traits. The two commercial cultivars of sunnhemp (K 12 yellow and K 12 black) were grouped in cluster-III, which comprised accessions with erect growth habit, fewer branches, less number of leaves, larger internode length and medium seed producers. In sunnhemp,

Table 5: Cluster mean values for different traits of sunnhemp accessions.

Characters	Cluster mean±SD			
	I	II	III	IV
DFF	49.39±1.81	46.23±3.30	44.82±3.18	33.44±0.51
PH	129.94±7.63	150.45±12.53	147.21±11.53	122.28±10.84
NLP	126.67±26.61	113.36±22.44	95.45±15.68	39.67±6.89
NPBP	9.08±3.15	9.21±2.28	6.75±1.49	3.83±0.50
NSBP	9.86±4.92	11.23±2.47	9.30±4.25	3.78±1.78
NIP	32.73±3.59	36.33±5.24	36.15±4.41	22.83±1.36
IL	2.43±0.29	2.31±0.35	2.62±0.25	3.16±0.21
NPP	69.45±13.3	97.09±26.18	68.02±18.67	47.89±1.71
NSP	10.41±1.35	11.13±2.11	9.00±1.53	7.50±1.37
SYP	14.38±2.72	22.06±4.42	14.55±3.23	9.21±0.85
HSW	3.11±0.33	3.42±0.29	4.01±0.38	4.41±0.32

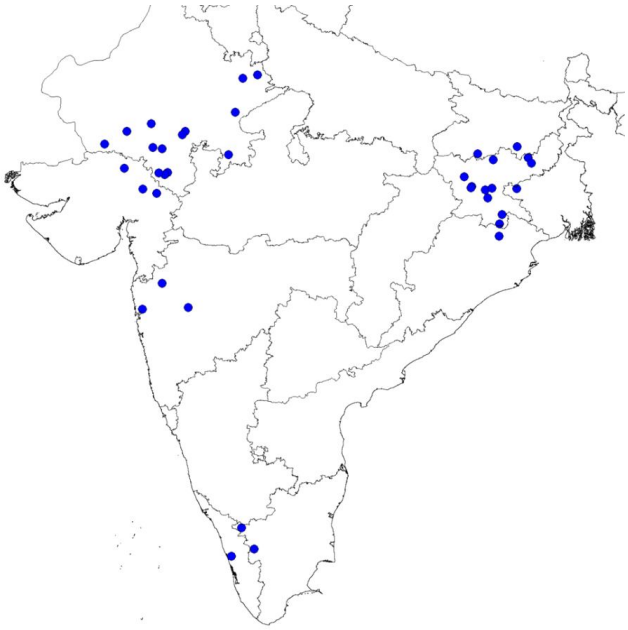

Fig 1: DIVA-GIS mapping of collection sites of sunnhemp germplasm from India.

seed yield is determined by the flower bearing ability of the plant which is greatly related to branching capacity of plant (Abdul-baki *et al*., 2001; Ulemale *et al.,* 2002; Cho *et al*., 2016). Since flowers of sunnhemp appear on the terminal racemes of primary and secondary branches, more the number of branches higher is the seed yield. Further branching has a positive effect on weed suppression which favors use of such variety as a cover crop. The study revealed valuable genetic resources of sunnhemp for varietal improvement. Therefore, accessions from cluster-I and II can be exploited in sunnhemp breeding program to develop a high seed yielding variety while maintaining all the beneficial qualities of a cover crop for enhanced biomass yield.

GIS mapping is an excellent tool for documentation and preliminary diversity analysis. Grid maps were generated for the diversity index for two important traits, NLP and SYP using DIVA-GIS software (Fig 3). High diversity index for number of leaves/plant was observed in the genotypes collected from parts of southern Rajasthan, Western Gujarat and Jharkhand regions. The highest diversity index for seed yield/plant was observed in accessions collected from central

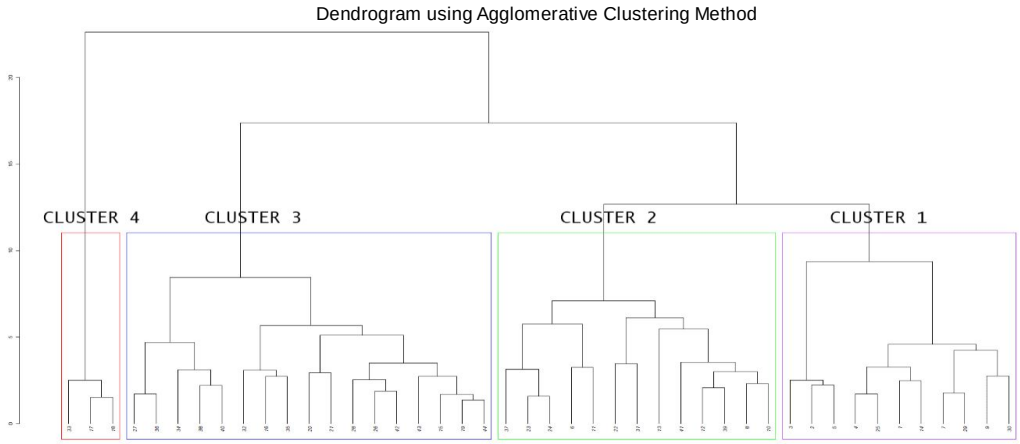

Fig 2: Dendrogram based on Euclidian's coefficient showing clustering of 44 sunnhemp accessions into four major groups.

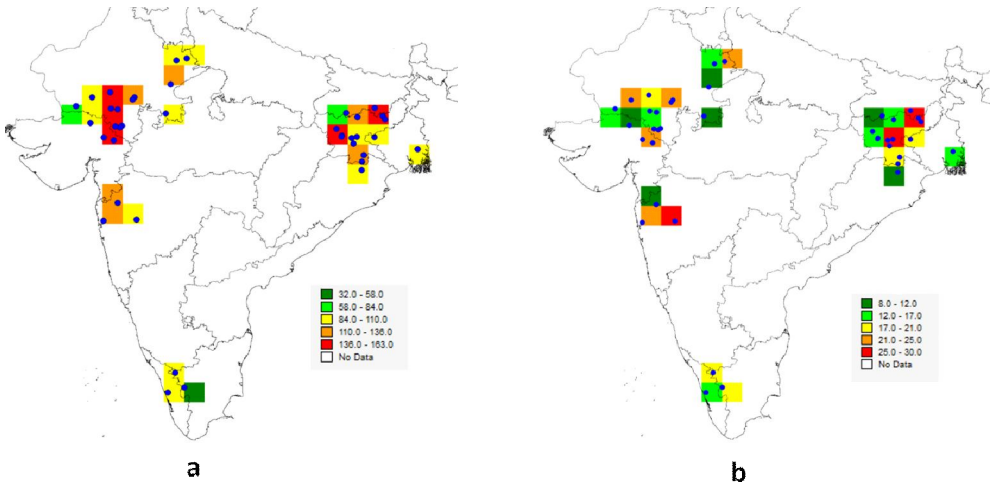

Fig 3:DIVA-GISgrid maps showing diversity index for (**a**) number of leaves/plant and (**b**) seed yield/plant in sunnhemp germplasm.

and northwest parts of Jharkhand and central parts of Maharashtra state. Grid maps further indicated that sunnhemp accessions collected from Tamil Nadu and Kerala recorded lowest diversity index for both the traits. The present study indicated the distribution of trait-specific diverse accessions of sunnhemp germplasm in various regions of the country and further enabled us to find out gaps in collection and diversity richness. Application of GIS mapping has been successfully used in the recent past in assessing the genetic diversity and in identifying areas of high diversity of different crops such as *Vigna mungo* L. Hepper (Abraham *et al*., 2010), *Kaempferia galanga* L. (Raina *et al*., 2015), *Linum usitatissimum* L. (Sivaraj *et al*., 2009) and medicinal plants (Varaprasad *et al*., 2007).

CONCLUSION

Overall, the present study highlighted substantial morphological diversity of sunnhemp accessions collected from diverse habitats belonging to a range of agro climatic conditions of India. Beside, heritability ascertained for important agro-morphological traits potentially important for diversified applications of the crop. Georeferencing these accessions together with highlighting hot spots for important traits provide baseline information to breeders for selecting potential parents in future sunnhemp breeding program. The information will prove enabler in identifying potential areas for future exploration program in the crop besides imparting fresh impetus on conservation measures to prevent potentially harmful anthropogenic activities in these regions.

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