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RESEARCH ARTICLE

Exploring genetic variability, diversity and trait associations in sunnhemp (*Crotolaria juncea* L.) accessions for yield improvement

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Abstract

Sunnhemp (*Crotolaria juncea* L.) is an important fibre crop known for its rapid growth and ability to improve soil fertility, making it a vital component of sustainable agriculture. However, to enhance its productivity and meet the increasing demand for high-quality fibre, it is crucial to identify and utilize genetically diverse genotypes with superior yield traits. The present study was conducted at the Tamil Nadu Rice Research Institute, Aduthurai, Tamil Nadu, during the *Kharif* seasons of 2021 and 2022 to assess genetic variability and character association for yield and its component traits among 67 sunnhemp genotypes. Multivariate analyses, including principal component analysis (PCA) and cluster analysis, were conducted using R software to dissect the phenotypic diversity among the genotypes. The results revealed substantial genetic variability for yield and its associated traits, indicating a promising potential for genetic improvement. Genotypes ADSH 1750, ADSH 1701, ADSH 1736, ADSH 1715, and ADSH 1742 exhibited superior performance in key yield-related traits, making them valuable candidates for future breeding programs focused on developing high-yielding, high-fibre varieties. Cluster analysis delineated four distinct clusters, with clusters I and IV showing significant divergence and highlighting the presence of unique genetic material. Key plant production traits such as plant height, leaf breadth, basal stem diameter, yield, and number of leaves were the primary contributors to the first two principal components. These findings suggest that direct selection based on these traits could effectively improve biomass yield in future sunnhemp breeding efforts, contributing to the advancement of sustainable fibre crop production.

Keywords

cluster; PCA; sunnhemp; Ward D^2

Introduction

Sunnhemp (*Crotalaria juncea* L.) is one of the oldest fibre species, widely distributed throughout tropical and subtropical countries (1). It is mostly grown for its delicate, slightly lignified stem fibres, are used for medicine, premium tissue, currency and cigarette paper (2-4). It is commonly grown on freshly reclaimed or fallow soils, where its main functions are to build or replenish the soil and repel nematodes (5, 6). In India, sunnhemp is mainly grown as a green manure crop, which is essential for maintaining soil health

(7).

Despite having numerous industrial applications, the area under sunnhemp cultivation has decreased dramatically, mainly due to lack of high performing cultivars (4). To address this issue, exploring and utilizing the genetic variability within sunnhemp genotypes is essential. Genetic variability is essential for any successful plant breeding program, as it provides the traits needed to improve crop performance, resilience, and yield (8). Identifying and using genetically diverse genotypes is crucial not only for increasing yield but also for ensuring the long-term sustainability of sunnhemp cultivation.

The main breeding strategies for fibre crops involve selecting cultivars that produce more fibre with better quality (9). However, the genetic resources of *Crotalaria* species have not been fully utilized. Although *Crotalaria* species are found worldwide, much of their genetic material has not been properly collected and conserved. There are over 600 species in the *Crotalaria* genus. However the USDA collection only contains only 242 entries (1), while in India, the Sunnhemp Research Station in Pratapgarh maintains only 122 different accessions, and the National Bureau of Plant Genetic Resources conserves around 400 accessions (10).

An understanding of genetic diversity is a prerequisite for any successful breeding programme. This study aims to assess the genetic variability and phenotypic diversity among 67 sunnhemp genotypes, focusing on yield and its component traits. Phenotypic diversity analysis is particularly important for revealing the extent of variability among the studied genotypes, which in turn provides insights into the potential for genetic improvement. Principal component analysis (PCA) is a powerful tool used to reduce the complexity of data sets, helping to identify key traits that contribute most to variability among genotypes (11). By analyzing the principal components, breeders can focus on the traits that have the greatest impact on yield and other desirable characteristics, making the selection process more efficient. Correlation analysis examines the relationships between different yield-related traits. Understanding these correlations is crucial for breeders, as it helps predict the outcomes of selecting for one trait over another (12). However, the genetic variability of Indian *Crotalaria* species is not well understood. To maximize biomass yield potential, this study was carried out to better understand of the genetic variability of sunnhemp genotypes.

Materials and Methods

A panel of 65 Sunnhemp (*Crotalaria juncea* L.) accessions, along with two check varieties, namely CO 1 and SH 4, was evaluated for yield and its component traits. The field experiment was conducted using a randomized block design at the Tamil Nadu Rice Research Institute, Tamil Nadu Agricultural University, Aduthurai, Tamil Nadu, India (10.99° N and 79.48° E). The study was carried out over two consecutive cropping seasons (April-June) in 2021 and 2022, with three replications to ensure robust results. The accessions were planted with a spacing of 30 cm × 5 cm in 3-meter-long rows.

Various agronomic and morphological traits were recorded, including days to 50% flowering (DFF, in days), plant height (PH, in cm), basal stem diameter (BD, in mm), number of leaves (NOL), leaf length (LL, in cm), leaf breadth (LB, in cm), and yield (in quintals per hectare, qtl/ha). The mean, minimum, maximum, and standard deviation of these traits were calculated using TNAUSTAT software (13) to assess the variation within the accessions. Pooled mean data from the two seasons were used for subsequent multivariate analyses. Correlation analysis, PCA and cluster analysis were performed using R software packages. Specifically, the “corrplot” package was used for correlation analysis, “FactoMineR” for PCA, “factoextra” for visualizing and interpreting the PCA, and “biotools” for cluster analysis (14-17).

Results

Variability in studied traits

The present study assessed 67 sunnhemp accessions, including two checks (CO 1 and SH 4), over two consecutive years (2021 and 2022) at the Tamil Nadu Rice Research Institute, Tamil Nadu Agricultural University. Significant variability was observed in the phenotypic traits measured. The Present study revealed a wide range of variability for the studied traits (Table 1). Accession ADSH 1717 was identified as the dwarf among the studied accessions in both years, while ADSH 1762 and ADSH 1701 were identified as the tallest accessions. ADSH 1737 (0.48 and 0.49 mm) was identified as having the highest basal stem diameters in both years respectively, whereas ADSH 1765 (1.12 and 1.15 mm) had the lowest basal stem diameter. Days to 50% flowering showed moderate variation. It ranged from 44 days (ADSH 1731) to 49 days (ADSH 1708) in 2021, while 45 days (ADSH 1715) to 50 days (ADSH 1761) in 2022. The minimum number of leaves was recorded in ADSH 1709 (36 and 37), while the maximum number of leaveswas observed in ADSH 1736 (90 and 91) in both years respectively. Leaf length ranged from 8 to 15.4 cm in 2021 and 8 to 16.3 cm in 2022, whereas leaf breadth ranged from 2.3 to 4.1 cm in 2021 and 2.2 to 4 cm in 2022. Biomass yield showed wider variation, ranging from 217.22 qtl/ha (ADSH 1760) to 489.50 qtl/ha (ADSH 1750) in 2021 and 232.22 qtl/ha (ADSH 1737) to 507.47 qtl/ha (ADSH 1701) in 2022. Accessions with high biomass yield, plant height and basal stem diameter are preferred, as these traits provides a high fibre recovery percentage, ultimately resulting in better fibre yield (18, 19). The accessions ADSH 1750, ADSH 1701, ADSH 1736, ADSH 1715 and ADSH 1742 recorded higher values for yield-associated traits. The observed variability in these traits underscores the broad genetic base present within the studied accessions. Thus, selecting these genotypes and utilizing them as donors in future breeding programmes could lead to the development of high fibre-yielding varieties.

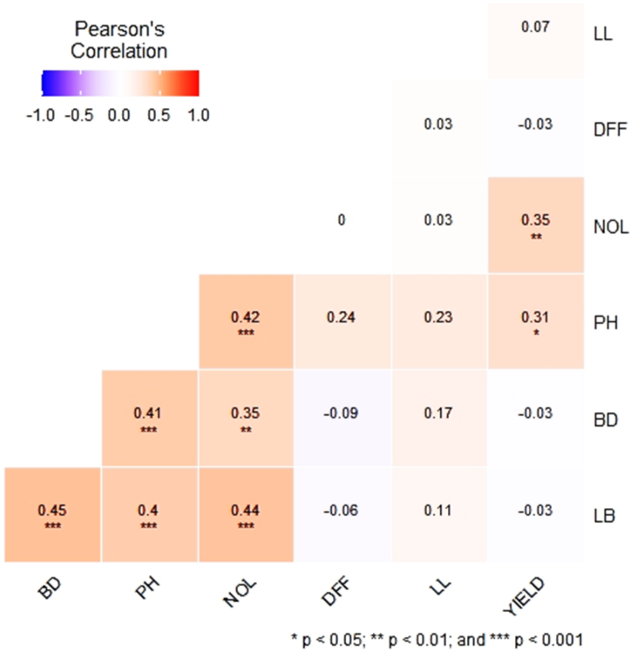
Table 1. Descriptive statistics of morphological traits studied

Traits	Season	Mean	SD	CV (%)	Min		Max	
					Value	Accession	Value	Accession
DFF	I	46.82	1.12	2.39	44	ADSH 1731	49	ADSH 1708
	II	46.99	1.18	2.51	45	ADSH 1715	50	ADSH 1761
PH	I	178.1	15.49	8.7	142.8	ADSH 1717	231.3	ADSH 1762
	II	177.44	14.86	8.37	145.4	ADSH 1717	225.6	ADSH 1701
NOL	I	64.7	10.2	15.76	36	ADSH 1709	90	ADSH 1736
	II	64.42	10.19	15.81	37	ADSH 1709	91	ADSH 1736
LL	I	12.32	1.86	15.12	8.0	ADSH 1754	15.4	ADSH 1729
	II	12.36	1.9	15.42	8.0	ADSH 1754	16.3	ADSH 1722
LB	I	3.32	0.38	11.5	2.3	ADSH 1719	4.1	ADSH 1740
	II	3.33	0.37	11.12	2.2	ADSH 1719	4.0	ADSH 1704
BD	I	0.83	0.13	15.86	0.48	ADSH 1737	1.12	ADSH 1765
	II	0.83	0.13	16.11	0.49	ADSH 1737	1.15	ADSH 1765
YIELD	I	350.38	58.45	16.68	217.22	ADSH 1760	489.50	ADSH 1750
	II	349.99	58.31	16.66	232.22	ADSH 1737	507.47	ADSH 1701

DFF-Days to fifty percent flowering, PH-Plant height, NOL-Number of leaves, LL-Leaf length, LB-Leaf breadth, BD-basal stem diameter.

Correlation analysis

The present research was undertaken to determine the associations of diverse traits for the contributing to biomass yield. Among the biometrical traits studied, the number of leaves exhibited the highest significant and positive (0.35**) association with biomass yield, followed by plant height (0.31**). Basal stem diameter exhibited a positive and significant association with biomass yield through plant height (0.41) and the number of leaves (0.35). Likewise, leaf breadth exhibited a positive and significant association with biomass yield through basal stem diameter (0.45), plant height (0.4) and the number of leaves (0.44). Results revealed significant positive associations between biomass yield and traits such as plant height, number of leaves, and leaf breadth. Specifically, taller plants with a higher number of leaves and broader leaves were associated with increased biomass yield. Thus, more emphasis should be placed on these traits during selection for high biomass yield. Additionally, basal stem diameter was positively correlated with plant height, suggesting that larger stem diameters could indirectly enhance yield through their association with height. The positive and significant correlation between the number of leaves and basal stem diameter further supports the importance of these traits in enhancing biomass yield. Basal stem diameter, the number of leaves and plant height were identified as the major contributing traits towards biomass yield (Fig. 1). This implied that yield enhancement could benefit from direct selection for these traits.



* p < 0.05; ** p < 0.01; and *** p < 0.001

Fig. 1. Association study of yield and its associated traits.

DFF-Days to fifty percent flowering, PH-Plant height, NOL-Number of leaves, LL-Leaf length, LB-Leaf breadth, BD-basal stem diameter

Cluster analysis

Cluster analysis using Ward's method classified 67 sunnhemp genotypes into four distinct clusters. Cluster III had the highest number of genotypes (29 genotypes), whereas cluster I had the lowest (6 genotypes). There were 20 genotypes in cluster II and 12 in cluster IV (Fig. 2).

Cluster I included genotypes (ADSH 1701, 1704, 1736, 1750, 1762 and 1706) with the highest mean values for plant height, number of leaves, and yield, making these genotypes particularly valuable for breeding programs focused on improving yield (Table 2). Cluster II contained genotypes with moderate plant height and yield, while cluster III comprised genotypes with high basal stem diameter and long leaves. Cluster IV contained genotypes with narrow leaves and fewer leaves, which may be less favorable for yield improvement but could possess other beneficial traits. The identification of distinct clusters helps in selecting appropriate parental lines for hybridization to develop high biomass-yielding varieties. Results revealed that Cluster I had higher mean values for the majority of the yield-attribute traits studied. Selecting high-yielding genotypes from cluster I for hybridization programmes could result in the identification of desirable and superior genotypes.

The Ward D^2 minimum variance method was utilized to estimate the intra-and inter-cluster distance. Of the four clusters, cluster I had the maximum (3.45) intra-cluster distance, while cluster II had the minimum (2.42) intra-cluster distance (Table 3). The maximum intra-cluster distance was found in cluster I, suggesting significant genetic variation among the genotypes which led to greater heterogeneity. The maximum inter-cluster

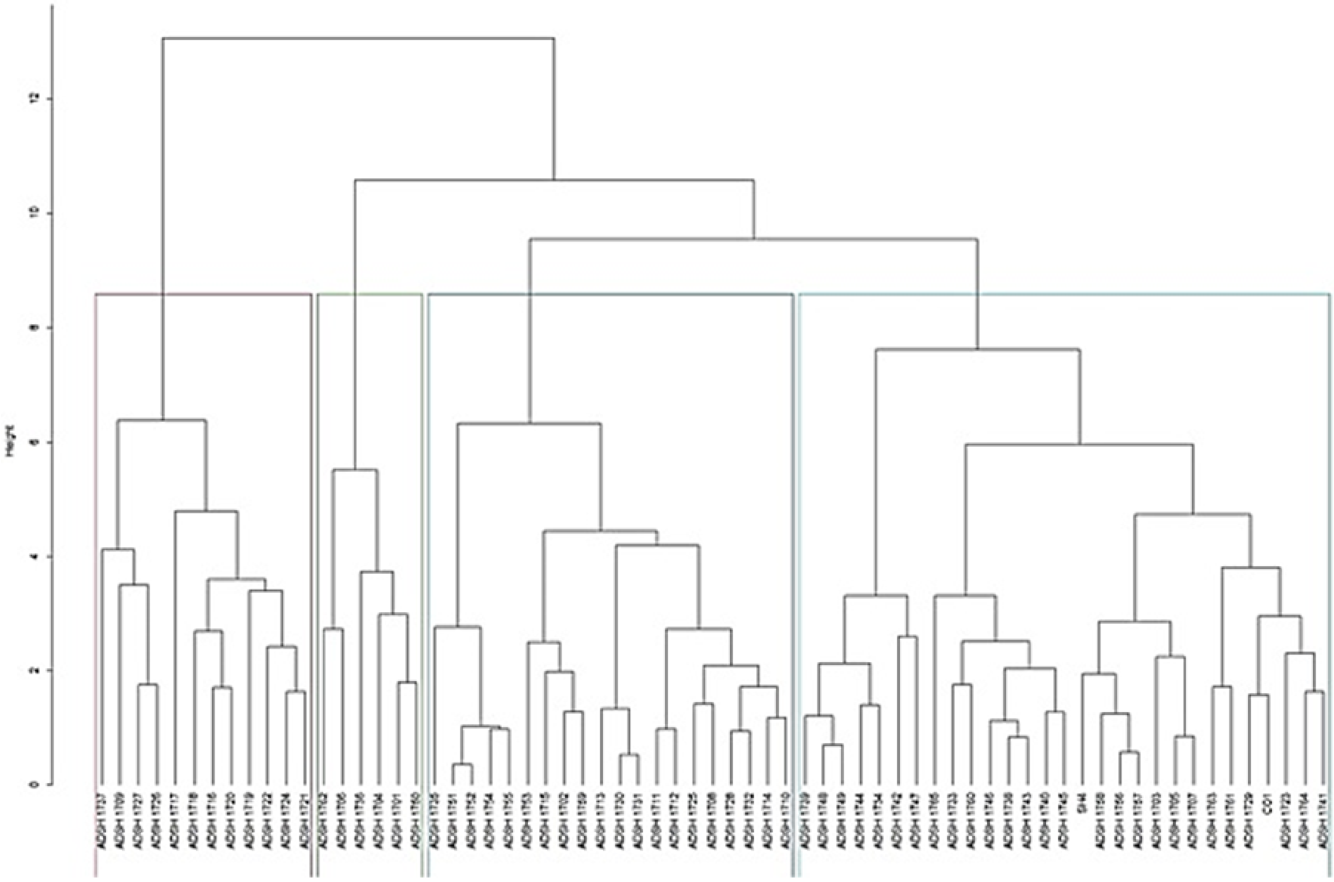


Fig. 2. Clustering pattern of studied accessions based on Ward D^2 minimum variance.

Table 2. Cluster means for morphological traits

Clusters	DFF	PH	NOL	LL	LB	BD	YIELD
I	47.17	208.53	81.17	13.40	3.50	0.95	432.33
II	46.80	172.99	67.65	10.55	3.41	0.77	364.80
III	46.83	178.65	65.10	13.57	3.47	0.89	333.34
IV	46.92	167.08	49.25	12.00	2.77	0.72	327.50

DFF-Days to fifty percent flowering, PH-Plant height, NOL-Number of leaves, LL-Leaf length, LB-Leaf breadth, BD-basal stem diameter.

Table 3. Intra and inter-cluster Euclidean distance values

Clusters	I	II	III	IV
I	3.45			
II	4.56	2.42		
III	4.30	3.19	2.72	
IV	6.21	3.92	4.20	3.42

distance was observed between cluster I and cluster IV (6.21), followed by cluster II (4.56) and cluster III (4.30). Cluster II which comprises 20 genotypes, was closer to cluster III (3.19), followed by cluster IV (3.92). Cluster III was away from cluster IV with a Euclidean distance of 4.20. Results implied that cluster I and IV were highly divergent, followed by cluster I and II. To obtains, novel desirable recombinants, genotypes in these clusters could be used for hybridization. Compared to individuals in other clusters, those grouped within the same cluster have closer relationships, implying that they are genetically less diverse among themselves.

Principal component analysis

Phenotypic data from 67 sunnhemp genotypes for seven morphological and plant production traits were utilized for principal component analysis. The first three principal components (PCs), which had eigenvalues>1, accounted for 66.56% of the total variability (Fig. 3). Other PCs (4 to 7) had eigenvalues less than 1. The first PC contributed 33.7% of the variation, while the second and third components explained 17.18% and 15.65%, respectively. The contribution of morphological traits to the total variation of each principal component is listed in Table 4.

For the first principal component traits, such as plant height, number of leaves, leaf breadth and basal stem diameter made major contributions to the 33.7% of total variability (Fig. 4). Similarly, yield, days to 50% flowering and basal stem diameter contributed to the total variation of 17.18% for PC 2, days to 50% flowering, yield and leaf

Table 4. Principal components of studied traits associated with biomass yield

PC components	PC1	PC2	PC3	PC4	PC5
Eigen value	2.35	1.20	1.09	0.95	0.56
% of variation	33.70	17.18	15.65	13.71	8.10
Cumulative % of variation	33.70	50.89	66.55	80.26	88.37
DFF	0.17	24.19	41.31	20.67	0.37
PH	25.95	6.08	3.72	0.24	7.71
NOL	23.29	1.17	10.68	3.75	12.24
LL	3.93	0.28	19.53	66.27	6.88
LB	21.45	14.05	0.09	3.28	27.47
BD	19.91	15.87	0.97	0.01	43.67
YIELD	5.31	38.36	23.69	5.79	1.66

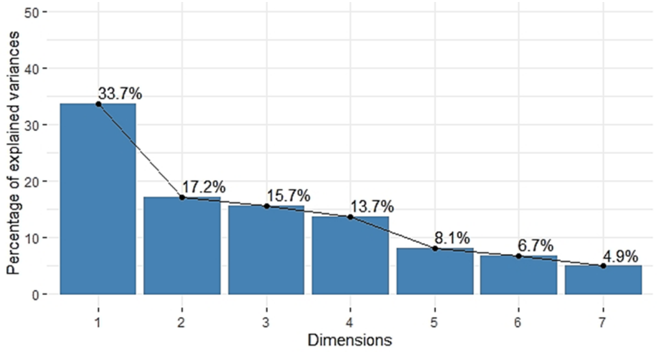


Fig. 3. Scree plot depicting Eigen values of PC components.

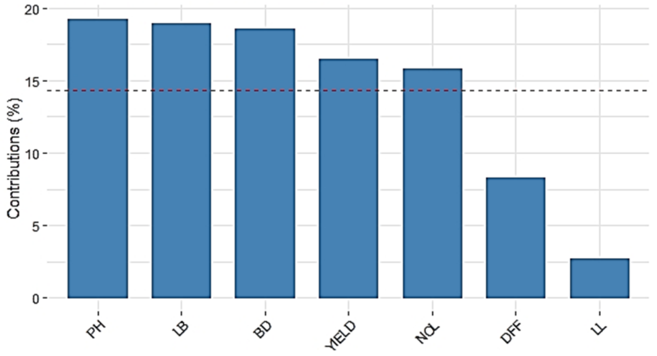


Fig. 4. Diagram depicting major contributing towards PC1.

length contributed to PC 3. Among the seven morphological and plant production traits, plant height, leaf breadth, basal stem diameter, yield and number of leaves had the majority contribution in the first two principal components. Consequently, the significant traits that contribute to variability in a particular PC tend to cluster together. Utilizing these traits in breeding programmes could enhance crop improvement.

The loadings for the seven traits studied in the PCA were broad, indicating the large diversity. Genetic diversity information is crucial for determining breeding tactics, heterotic grouping, and forecasting the performance of hybrids. The distribution and degree of diversity for both the accessions and the traits studied were depicted in the biplot (Fig. 5), which shows the relationship between PC1 and PC2. Almost all accessions and traits studied showed a great degree of variance. All fourquadrants comprised of an average of 17 genotypes. All the genotypes were clustered into 3 different clusters based on the principal components. The trait days to 50% flowering had a major contribution towards cluster I while yield and leaf length contributed to cluster III. The traits such as plant height, number of leaves, leaf breadth and basal stem diameter were major contributors to cluster II.

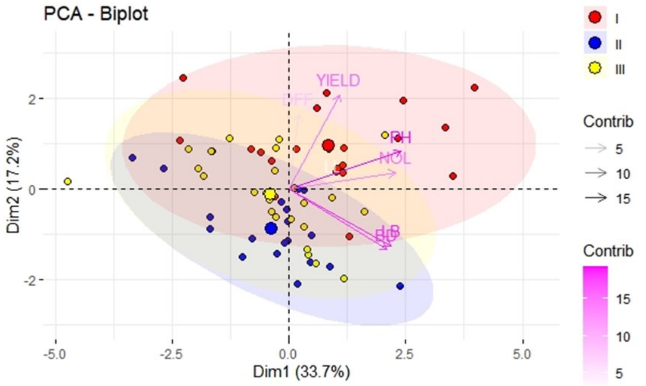


Fig. 5. PCA Biplot analysis of sunnhemp accessions for PC1 *vs* PC2 components.

Discussion

Biomass yield in sunnhemp is a complex trait influenced by both plant characteristics and environmental factors (20). Thus, correlation coefficient analysis is frequently used to assess the strength and direction of the association between various plant attributes and biomass yield (21). Results revealed significant positive associations between biomass yield and traits such as plant height, number of leaves, and leaf breadth. Specifically, taller plants with a higher number of leaves and broader leaves were associated with increased biomass yield. Thus, more emphasis should be given to these traits during selection for high biomass yield. These findings are consistent with previous studies indicating that these traits contribute to higher biomass production (18-20). Additionally, basal stem diameter was positively correlated with plant height, suggesting that larger stem diameters could indirectly enhance yield through their association with height. The positive and significant correlation between number of leaves and basal stem diameter further supports the importance of these traits in enhancing biomass yield (20).

Cluster I included genotypes (ASDH 1701, 1704, 1736, 1750, 1762 and 1706) with the highest mean values for plant height, number of leaves, and yield, making these genotypes particularly valuable for breeding programs focused on improving yield. Cluster II included genotypes with moderate plant height and yield, while Cluster III was consisted of genotypes with high basal stem diameter and long leaves. Cluster IV contained genotypes with narrow and fewer leaves, which may be less favorable for yield improvement but could possess other beneficial traits. The identification of distinct clusters helps in selecting appropriate parental lines for hybridization to develop high biomass-yielding varieties (22, 23). Maximum intra-cluster distance was found in cluster I, suggesting that there may be significant genetic variation among the genotypes which, led to more heterogeniety (22, 23).

Among the seven morphological and plant production traits, plant height, leaf breadth, basal stem diameter, yield and number of leaves made the largest contributions in the first two principal components. Consequently, the significant traits that contribute to variability in a particular PC tend to cluster together. Utilizing these traits in breeding programmes could enhance crop improvement (24). The loadings for the seven traits studied in the PCA were broad, which shows the large diversity. Genetic diversity information is crucial for determining breeding tactics, heterotic grouping, and forecasting the performance of hybrids (25).

Conclusion

Current study demonstrated that there is significant genetic variability among 67 sunnhemp accessions, which is important for breeding programs focused on improving biomass yield and fibre quality. Key traits, like plant height, number of leaves, and basal stem diameter, as well as distinct groups of genotypes were identified, providing a solid basis for selecting high-yielding, fiber-rich sunnhemp cultivars. Principal component analysis highlights the importance of these traits in contributing to overall genetic diversity. The findings suggest that these diverse accessions can be used to develop high-yielding, fibre-rich sunnhemp cultivars, which will help improve the crop's industrial uses and sustainability.

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Authors' Contributions

RP contributed to conceptualization and investigation. RP and RA were involved in project administration and writing—review and editing. AS and RA conducted experimental analysis, formal analysis, methodology, data curation, and writing original draft. DS, RS, and KS contributed to methodology, validation, and writing—review and editing. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used AI tool- QuillBot to improve language and grammar. After using this tool, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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