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Genetic Divergence Studies among Indigenous Accessions of *Cassia auriculata* (L.) *Roxb*., through Screening and Utilization for Chambal Ravine Rehabilitation

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ABSTRACT

Background: *Cassia auriculata,* L., is an imperative medicinal and traditional neutral colour leaf hair dye yielding in India. This is a multipurpose and indigenous, perennial leguminous shrub and widely used as traditional medicine to cure diabetics by the rural peoples. Tanner's cassia is also used as neutral henna and its yields natural golden blonde colouring to hair through the dried leaf powder. Young buds are used for yellow colour dye extraction by textile industries. Screening of high tolerance and best performing genotypes is highly essential in this imperative woody legume shrub to rehabilitate the mass erosion prone gullied and ravine lands for productive purpose of the wasteland and degraded lands.

Methods: Thirty different accessions of *Cassia auriculata* were assembled and numbered from CA-1 to CA-30 from arid and semi-arid regions of Rajasthan (India). The experimental trial was conducted at ICAR-IISWC, Research Centre, Kota-Rajasthan, India during 2019-2022. After evaluation, CA-4 genotype was selected as an elite genotype. The elite genotype was used for high density plantation development with resource conservation for green cover development in non-arable ravine lands.

Result: Genetic variation studies of *Cassia auriculata* indicated that considerable and significant variability, abundant morphometric variations in assembled genotype progenies. Further, genetic divergence analysis was also revealed that nine clusters segregated from 30 genotypes. Thirty genotypes were grouped into nine clusters according overall performance with geographical identity. Finally, *C. auriculata* (CA-4) genotype was identified and selected as an elite genotype based on its overall superiority in terms of plant growth, higher green biomass, leaf litter biomass, more number branching behaviour and plant coppicing ability which are directly relevant and imperative in soil erosion control, resource conservation and potential rehabilitation effect in resource poor dry regions of non-arable lands.

Key words: Genetic diversity, Neutral Henna, Phenotypic Variation, Rehabilitation, Tanner's Cassia, Tannin and woody legume.

INTRODUCTION

Perennial woody coppicing plants or shrubs are act as a potential barrier to soil erosion, water runoff, increase insitu moisture regime, particularly in drylands and sometimes act as inexpensive life fences. A major reason for adopting legume shrubs in dryland forestry and agroforestry farming systems for effective utilization lesser know multipurpose leguminous shrubs specifically for degraded land management and for enhancing soil productivity. Many times, multipurpose shrubs are a long-neglected life form in the plant community, but it provides wide array of economic and environmental benefits to human and animal society (Banerjee 1989). It is a hardy multipurpose leguminous shrub belongs belonging to *Fabaceae*, it is globally or commonly known as *Tanner's cassia*. Avartaki (*Cassia auriculata* (L.) Roxb. (Syn. Senna auriculata) is an important herbal drink in entire South Asian countries. All parts of plant parts of *C.auriculata* have been used traditionally in ayurveda, siddha and Unani since the 15th century. It provides the limelight for the traditional claims and its medicinal uses in various ailments since centuries in folkloric practices (Nile *et al*., 2021).

Considering higher demands of plant based medicines and dyes, flower and leafy biomass as raw material for many pharmaceutical and agro-industries through heavy and unregulated wild collection of *cassia auriculata* resources may create threatening to natural genetic resource. It is a small bush, which grows wild in South India with flowers

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and pods throughout the year reported by Mathew, 1983. The species has widespread distribution in entire semi-arid regions of Rajasthan with enormous variability in its growth, form, vigour, flower colour, fruit shape and seed yield. This good quality fuelwood species with high calorific value; tolerant to annual coppicing, has golden yellow cluster flowers and produces hard seed coat seeds. Plant propagation is commonly practiced by seed. *Cassia auriculata* is one of the most important natural dye yielding cum medicinal shrub in drylands of India. It grows up to a height of 60 to 150 cm. It has been widely used in Ayurvedic medicine as “Avarai Panchaga Choornam” and the main constituent of Kalpa herbal tea has come under extensive study in the light of its anti-diabetic effects reported by Pari and Latha, (2002). *Cassia auriculata*, L. Roxb., inn is also yields natural golden hair dye through leaf powder. Thus, it can be utilized for natural dye extraction and being potential legume shrub for eco-restoration purpose.

Morphological uniqueness considered as a first step in plant diversity assessments for use of plant genetic resources by Mwanga *et al.* (2017). Morphometric traits were reported as useful tools to capture leaf shape and leaf colour information and their accuracy to improve morphological characterizations and contribute effectively with accurate data in genetic studies. Individual accessions /genotype research is therefore of paramount importance. The accessions collected are significant source of gene complex reservoir for economically an important trait as they consist of untapped genetic pre-requisite in formulating efficient plan of genetic and crop improvement studies (Shekhawat *et al*., 2023). The most common, easy to handle and quick gains can be achieved in long rotation perennial species with use genotype selection within species by Zobel and Talbert (1984). Therefore, present investigation has been carried out to estimate genetic variation present in *Cassia auriculata* populations. Inspite of such a varied medicinal uses and ecosystem value, no improved germplasm/genotypes are not available in this species. The extent of variability identified gives better scope to breeders for selection. So, using D^2 statistic, it can determine the grouping pattern of selected genotypes into different clusters according to their genetic similarity. Since, research work on genetic diversity and multivariate analysis is lacking in this species, the present study was carried out to scrutinize the nature and magnitude of genetic variability and diversity in the natural population or accession of *C. auriculata*.

MATERIALS AND METHODS

An intensive survey made in semi-arid regions of Rajasthan based on candidate genotype selection method or comparison check tree method (selection criteria) developed by Zobel and Talbert (1984). Accordingly, thirty indigenous genotypes of *Cassia auriculata* were collected from different geographical locations which were given in Table 1 and Photo 1. The seeds of superior genotypes were used in the study and it was conducted ICAR-IISWC, Research Centre, Kota-Rajasthan, India during 2019-2022 (Table 1). The mean values of all biometric data were subjected to analysis of variance (ANOVA) to establish the significance of differences between the genotypes. Data were analysed statistically according to method described by Panse and Sukhatame (1978). Phenotypic and genotypic variances were estimated as per the method described by Johnson *et al*. (1955a). Phenotypic and genotypic coefficients of variances (PCV and GCV) analysis were computed following Burton (1952). Broad heritability (h^2) was calculated according method given by Lush (1940). Genetic advance was worked out based on Goulden (1952). Mahalanobis (1936) D^2 multivariate analysis conducted with selected genotypes level. The accessions were grouped into clusters following Tocher’s method inter cluster D^2 and cluster wise mean values were also estimated.

RESULTS AND DISCUSSION

In this study, a total of seven biometric traits were considered for evaluation and significant variation were observed among the progenies performance for shoot length, root length, collar diameter, number of leaves, dry weight, quality index and volume index at standard intervals after sowing which indicated that substantial amount of genetic variability existed among the 30 genotype progenies. Mean performance genetic parameter estimates of growth traits are essential for the breeder to identify superior genotypes. The spectacular variation in plant growth and morphological traits like flower and pod variation features were observed among the assembled progenies in this study is attributed to its genetic variability (Photo 1). The morphological traits of the all accessions indicated wide variability for all the characters studied and hence ample opportunities exists for genetic improvements of the crop through selection directly from the genotypes for improvement programme three genotypes *viz*., CA-4, CA-3 and CA-1 showed consistent superior performance for all the biometric traits (Table 2 and 3). Variations in growth attributes due to progenies and provenances were also earlier reported in progenies of Neem (*Azadirachta indica*) was reported by Dhillion *et al*. (2007) and in the progenies of Kachnaar (*Bauhinia variegata) by* Thakur *et al.* (2009).

In variability studies, volume index had recorded high phenotypic and genotypic coefficient of variation of 27.29 and 26.22 followed by shoot length (20.58 and 19.37), total dry weight (13.05 and 12.82), sturdiness quotient (12.77 and 11.20), number of stems/plant (10.38 and 8.15), root length (10.58 and 9.56) and (Table 4). Volume index recorded heritability of 92.35 and high genetic gain as percentage of mean was 51.91. This was followed by plant dry weight recorded highest heritability of 96.61 and genetic gain as percentage of mean of 25.96 (Table 3). Hence, selection based on phenotypic performance of traits would be more effective in genotypes. Therefore, all recorded values were highly influenced by the genetic makeup individual plant. It was a true reflection of inherent additive

Table 1: Details of about thirty assembled indigenous accessions of *Cassia auriculata.*

Place of collection and accession name	Taluk/Tehsil	Latitude	Longitude	Plant height (m)	Plant collar diameter (cm)
Jhalawar-CA-1	Jhalawar	24.59°N	76.16°E	2.65	16.50
Jhalawar-CA-2	Jhalawar	24.59°N	76.16°E	3.05	19.15
Eswal-CA-3	Rajasmand	25.06°N	73.89°E	2.68	17.30
Eswal-CA-4	Rajasmand	25.06°N	73.89°E	2.15	15.50
Sajangarh-CA-5	Udaipur	25.57°N	73.70°E	2.45	18.50
Mauvili-CA-6	Udaipur	25.57°N	73.70°E	2.10	17.10
Chittorgarh-CA-7	Chittorgarh	24.88°N	74.63°E	2.55	22.60
Kumbalgarh-CA-8	Rajasmand	24.15°N	75.58°E	2.60	18.12
Jaisamand-CA-9	Udaipur	23.91°N	73.41°E	2.35	20.10
Bagdhara-CA-10	Banswara	23.54°N	74.43°E	2.06	15.20
Sivagangai-CA-11	Tamil Nadu	9.84°N	78.48°E	2.75	17.10
Bisalpur-CA-12	Pali	25.77°N	73.33°E	2.18	18.10
Balwana-CA-13	Pali	25.69°N	73.41°E	1.75	21.70
Perwa-CA-14	Pali	25.47°N	73.37°E	2.40	20.15
Semerpur-CA-15	Pali	25.97°N	73.33°E	2.20	18.45
Gatla-CA-16	Jaisamand	23.95°N	73.46°E	2.28	15.12
Retguriya-CA-17	Jaisamand	23.95°N	73.46°E	2.70	13.50
Veerpura-CA-18	Jaisamand	23.95°N	73.46°E	2.10	16.15
Pilader-CA-19	Jaisamand	23.95°N	73.46°E	2.42	22.10
Khanakheri-CA-20	Jaisamand	23.95°N	73.46°E	1.70	14.50
Kewalpura-CA-21	Chittorgarh	24.32°N	74.43°E	2.15	20.35
Jarkharana-CA-22	Chittorgarh	24.32°N	74.56°E	2.40	19.50
Salumber-CA-23	Salumber	24.13°N	74.03°E	2.58	17.10
Bhagatpura-CA-24	Banswara	23.56°N	74.44°E	1.90	16.40
Janawari-CA-25	Banswara	23.56°N	74.44°E	2.25	20.60
Kanpuri-CA-26	Banswara	23.56°N	74.44°E	2.40	16.12
Kesarpura-CA-27	Banswara	23.56°N	74.44°E	2.85	22.10
Kherwara-CA-28	Dungurpur	23.83°N	73.47°E	2.26	18.20
Antri-CA-29	Dungurpur	23.83°N	73.47°E	2.35	16.60
Rampur-CA-30	Dungurpur	23.83°N	73.47°E	2.28	18.10

Table 2: Analysis of variance for biometric traits of *Cassia auriculata.*

Source	d.f	Mean squares values							
		Shoot length	Root length	Collar diameter	No. of stems	Root/shoot ratio	Total dry weight/plant	Quality index	Volume index
Total	145	23.68	11.95	0.24	2.06	0.012	4.35	0.128	1692.66
Genotypes	29	102.60**	43.26**	0.458**	7.85*	0.171*	21.65**	0.180**	12419.24**
Error	116	4.54	4.35	0.063	0.53	0.034	0.15	0.029	0.864
SE.d		1.34	1.32	0.035	0.85	0.022	0.24	0.02	0.026
CD (0.05)		3.51	3.45	0.09	1.82	0.047	0.64	0.05	0.058

*Significant at five per cent; **Significant at one per cent.

Table 3: Genetic parameters for biometric traits.

Characters	Phenotypic variance	Genotypic variance	PCV (%)	GCV (%)	Heritability (%)	GA as % of mean
Shoot length	24.03	19.65	10.58	9.56	81.76	17.81
Collar diameter	0.012	0.010	7.54	6.97	85.32	13.26
Root length	11.39	7.97	10.98	9.56	69.98	11.30
Number of stems/plant	22.06	13.85	10.38	8.15	61.76	13.20
Total dry weight	4.452	4.301	13.05	12.82	96.61	25.96
Sturdiness quotient	0.004	0.003	12.77	11.20	76.87	20.22
Root/shoot ratio	703.99	650.14	27.29	26.22	92.35	51.91

gene effect. Similar kind of PCV and GCV studies were also reported in *Pongamia pinnata* by Vennila *et al.* (2022).

The clustering pattern indicates that diversity was not related to their geographical distribution of trees, as the plus trees of a particular region were spread over all the clusters. Thus the geographic diversity cannot always be used as an index to genetic diversity as advocated by several research workers by Sehgal *et al*., (2003). On the basis of Mahalanosis D^2 analysis, 30 genotypes were grouped into nine clusters. The distribution of genotypes in different clusters varied from I to IX (Fig 1). Cluster I was largest which accommodated seven genotypes *viz*., CA-8, CA-20, CA-10, CA-17, CA-23, CA-24 and CA-26. The clusters II, V, VII and IX were constituted two progenies each. The clusters III and VI were constituted by three progenies each. Finally, cluster VIII was constituted by five progenies *viz*., CA-22, CA-21, CA-28, CA-18 and CA-15. Cluster IV was distinct and unique from others as it included four progenies. On contrary, the progenies from clusters I, II, III, IV, VII and VIII included selections from all the locations indicating adequate genetic variability among the clusters under this study.

The clustering patterns also revealed that the tendency of progenies from diverse geographic region to be grouped together in one cluster may be due to the similarity of the nature of selection pressure operating under respective domestic conditions. The present study results indicated that factors other than geographical divergence may be responsible for the different grouping of progenies (Fig 1). Further the results agreed with the findings reported by Bagchi (1992) in *Acacia nilotica* that the genetic proximity was not dependent on geographical nearness. Similar kind of clustering studies among provenance was observed in *Gliricidia sepium* by Rajaram (1990) and Manasa *et al*., (2022). The intra and inter cluster distance are presented in Table 4. The average intra-cluster distance was maximum (78.66) in cluster VIII followed by cluster VI (63.28) and cluster I (50.36), revealed the existence of diverse genotypes / trees in these clusters. Minimum intra-cluster distance (0.000) was shown by cluster IX having four genotypes. The inter-cluster D^2 values ranged from 2.19 to 198.33, the maximum value between cluster IV and cluster VIII (198.33) followed by cluster I and VIII (157.33), cluster VII and VIII (142.97) indicating maximum divergence between the genotypes included in these clusters, hence they are utilized in further breeding programme. Hybridization between genotypes from such clusters may help to develop promising

Table 4: Intra (diagonal) and inter cluster distance D^2 and D (Parenthesis) of the progenies.

Clusters	I	II	III	IV	V	VI	VII	VIII	IX
I	50.36	32.27	30.98	32.02	49.35	39.49	63.99	157.33	41.33
II		4.88	0.861	2.19	10.33	13.44	31.76	122.00	14.54
III			21.54	5.94	29.91	5.94	53.48	56.44	21.89
IV				51.65	12.96	8.92	35.88	198.33	18.16
V					31.96	32.46	24.88	46.68	19.36
VI						63.28	7.065	142.97	35.21
VII							45.56	72.67	31.89
VIII								78.66	40.63
IX									0.000

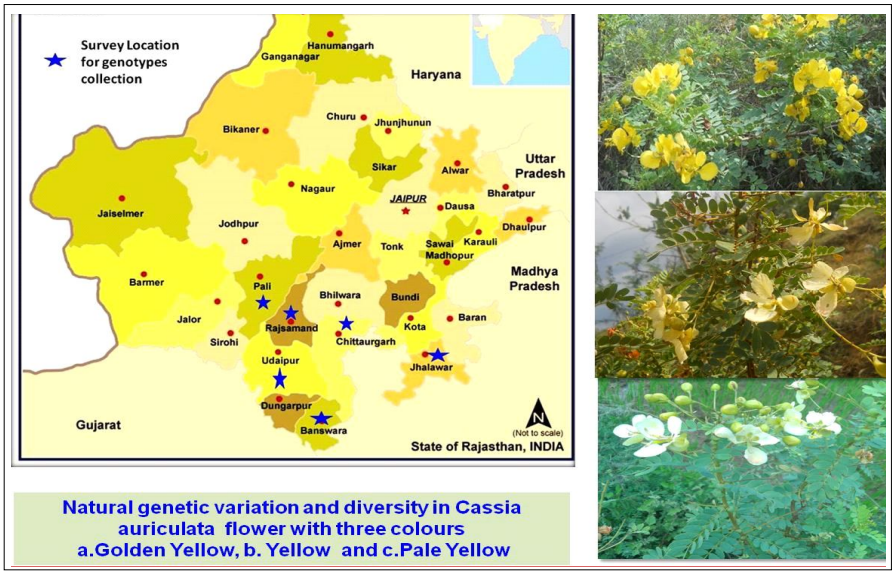
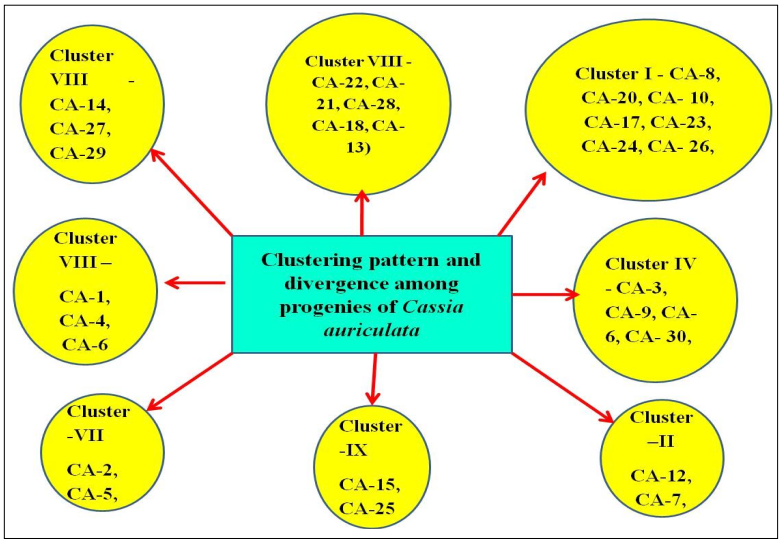


Photo 1: Location map indicating place of collection and natural genetic variation in flower colour of *Cassia auriculata*.



Cassia hybrids and/or can yield fruitful segregants. The results of present study are in line with the findings of other workers in different tree species *viz*., *Bauhinaia variegata* by Anand *et al*. (2005) in *Azadirachta indica* by Kaushik *et al*. (2007) and in *Bixa orellana* by Kala and Kumaran (2012). The minimum inter-cluster distance (2.19) between cluster II and IV revealed that the trees belonging to these clusters were genetically closer. The maximum inter-cluster distance was observed between cluster IV and VII (Table 4). The maximum intra-cluster distance was shown by cluster VIII. Hence, the clusters were genetically divergent from each other. The clustering pattern had no relationship between the genetic divergence and geographical distribution. Therefore, hybridization involving genotypes of clusters IV and VII is recommended in order to achieve high yielding varieties in *Cassia auriculata*.

CONCLUSION

Cassia auriculata is directly used medicinal plant popularly by diabetic's patients as a supplementary medicine since centuries. Present investigation is envisaged that seed sources *viz*., CA-4, CA-3 and CA-1 were expressed superiority over others for all the biometrical characters used in the progeny evaluation trials. Volume index recorded heritability of 92.35 and high genetic gain as percentage of mean was 51.91. High and positive association coupled with intensive direct effect of plant height followed by collar diameter and number of branches could be used as valuable, reliable and relevant yardsticks for selection in crop improvement programme. These thirty genotypes were grouped into nine clusters. The average intra-cluster distance was maximum (78.66) in cluster VIII followed by cluster VI (63.28) and cluster I (50.36), revealed the existence of diverse genotypes/trees in these clusters. There is no relationship were observed between clusters and it indicated inherent distance in genetic divergence and geographical distribution. Cluster-VII genotype was most divergent as well as had higher means for all seedling quality traits which are economically valuable in this study. The identified Cluster VII were considered as the superior genotypes and their popularization among the interested and commercial growers is highly needed and essential to meet the demand of fresh and dry flowers, green cover development in drylands, livelihood generation and it is highly essential for genetic resource conservation for further crop improvement of this high valued medicinal plant.

Conflict of interest: Nil.

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