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Research Article

Seed morphological characterization, genetic diversity and association analysis in late flowering monoecious lines of castor (*Ricinus communis* L.)

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Abstract

Castor is an important industrial oilseed crop utilized for multivarious applications. Augmenting seed yield in long duration castor varieties is of utmost importance to meet the increasing oil demand and also to make them suitable for inter/border cropping. Understanding the genetic diversity in base population of monoecious lines and interrelationship between economic traits are very much important to formulate suitable genetic improvement programmes. In this regard, seed morphological characterization and genetic diversity was assessed in 62 late flowering monoecious lines. Predominance of brown colour, ellipsoid shape and conspicuous mottling with caruncle was observed among the genotypes. Cluster analysis grouped them into eight different clusters. Maximum inter cluster distance was found between cluster VI and VIII, whereas intra cluster distance was high in cluster II. Cluster VI recorded the highest cluster mean for 100 seed weight and seed yield per plant (44.56 g and 174 g, respectively). Association studies revealed 100 seed weight and shelling percentage to be the significant traits for indirect selection of yield. In conclusion, the cluster VI has potential genotypes to utilize in developing high yielding varieties and male parent donor for hybrid breeding. The diverse genotypes identified from this study could be given priority during recombination breeding to get diverse segregants and for further molecular analysis like mapping population development and QTL/gene identification.

Keywords: Castor, Monoecious lines, Diversity, Correlation, Path analysis

INTRODUCTION

Castor (*Ricinus communis* L.), a diploid monotypic member of the family Euphorbiaceae is well adapted to tropical, subtropical and temperate regions. It is an allogamous crop due to its monoecious and protogynous nature (Allan *et al.*, 2007; Moshkin, 1986). India is leading the world in production and export of castor oil which has wide and varied use including biofuel (Sadaiah *et al.*, 2021). Due to its multifarious industrial

applications, the demand for castor oil in international markets has raised considerably (Patel *et al.*, 2018). Seed, the economic part contains approximately 45-55% oil, the major portion of which is ricinoleic acid, an unusual hydroxyl fatty acid (Rukhsar *et al.*, 2017). Increasing the percentage of oil content to a significant amount is tedious and hence, production and productivity should be improved to meet out the oil demand. For doing so,

weightage should be given to seed related metric traits. Castor genotypes are classified as early (<65 days) and late flowering (>65 days) based on duration taken from sowing to 50 % flowering (<https://www.plantauthority.gov.in/crop-dus-guidelines>). Castor is normally cultivated as a six-month crop with multiple harvest starting from the third month. It is highly suitable for inter cropping/ border cropping with legumes and shade cropping in turmeric and banana. In this regard, late flowering genotypes were given importance for developing long duration castor varieties.

In India most of the existing diversity is concentrated in varied ecosystems (Anjani, 2012). Diversity among the genetic material to be used as parent is of utmost importance in any castor breeding programme. Progenies derived from such diverse crosses are likely to express a broad spectrum of genetic variability, thus increasing the scope for the isolation of transgressive segregants in advance generation and also expected to express a higher magnitude of heterosis (Patel *et al.*, 2018). Hence, the present study was undertaken to study the diversity and character association for seed related quantitative traits among late flowering monoecious lines of castor.

MATERIALS AND METHODS

Genetically pure seed material of 62 castor genotypes which includes local landraces collected from Tamil Nadu, germplasm accessions, inbred lines, varieties, advanced breeding lines were sourced from Tapioca and Castor Research Station, Yethapur, Tamil Nadu Agricultural University. The genotypes were raised in Randomized Block Design with two replications during *Rabi*, 2020 with the plot size of 3.6 m² per replication. The experimental station is located between 11° 35' N latitude and 78° 29' E longitude at an altitude of 282 m above mean sea level. The spacing adopted was 90 × 90 cm and the recommended agronomic packages of practices were followed to facilitate healthy crop growth.

Harvesting was done at maturity stage at different intervals and data were observed for qualitative traits viz., presence of secondary coloration (PSC), main seed coloration (MSC), secondary coloration of seeds (SCS), type of secondary coloration (TSC), seed shape (SS) and caruncle protuberance (PDC)] and quantitative traits [days to 50% flowering (days), seed length (cm), seed breadth (cm), length/breadth ratio, 100 seed weight (g),

shelling percentage (%) and seed yield per plant (g) as per the descriptors suggested by Ministry of Agriculture, Livestock and Supply (Mapa) and Center for Genetic Improvement and Biotechnology Program (NBIO) of the Federal University of Reconcavo da Bahia (UFRB), Cruz das Almas-BA, Brasil (Silva *et al.*, 2019). Seed morphological traits plays an important role in germination and acts as an oil precursor (caruncle), mottling pattern (alkaloid poisoning), round shape with darker outer shell (irradiation tolerance). Hence, the characters under study will be helpful in yield/economic breeding of castor. Data were recorded on three randomly selected plants in each replication and the mean values were subjected to cluster, correlation and path analysis. Statistical analysis was done using R software version 4.1.1. Cluster analysis was carried out based on Euclidean distance using Ward's method. Inter and intra cluster distance and cluster mean were computed and the optimum number of clusters were found using NbClust package available in the software. Correlation and path analysis were carried out to elucidate the true picture of association among the characters studied.

RESULTS AND DISCUSSION

Characterization based on phenotypic traits is the most important process in assessing the diversity among any genetic material (Mwanga *et al.*, 2017). Analysis of variance (ANOVA) revealed the existence of sufficient variation among the genotypes studied (**Table 1**). Late flowering monoecious lines of castor is significant while developing varieties/hybrids suitable for intercropping/border cropping. Yamanura and Mohan (2020) also suggested that medium to late maturing genotypes should be exploited to augment higher seed yield in castor for intercropping and shade cropping. Kumar *et al.* (2022) stated that early varieties were low yielding and hence, emphasis had to be given to long duration varieties. Hence, the genotypes were selected based on days to 50% flowering which produces inflorescence after 65 days from sowing as per standard DUS guidelines (<https://www.plantauthority.gov.in/crop-dus-guidelines>). Morphological description of 62 late flowering genotypes for six seed based qualitative traits are given in **Table 2**. While observing the seeds, all the genotypes possessed the secondary color which was mostly white. Primary color of the seeds varied considerably among the seed material where they possessed different shades of brown ranging from light

Table 1. Analysis of variance (ANOVA) for biometrical traits in castor

Source	DF	Days to 50 % flowering	Seed length	Seed breadth	L/B ratio	100 seed weight	Shelling percentage	Seed yield per plant
Replication	1	0.516	0.002	0.028	87.052	31.00	124.00	31.020
Genotype	61	33.020***	0.039***	0.174***	0.016	78.080***	291.420***	3328.30***
Error	61	0.385	0.001	0.001	0.020	0.320	0.002	0.040

***p<0.001

Table 2. Phenotypic description of six categorical traits in late flowering monoecious lines of castor

S.No.	Genotypes	PSC	MSC	SCS	TSC	SS	PDC
1.	MI 158	Present	Medium brown	White	Scored	Ellipsoid	Slight
2.	MI 159	Present	Medium brown	White	Scored	Ellipsoid	Slight
3.	M3/28-3-1	Present	Dark brown	White	Cracked	Ellipsoid	Prominent
4.	DCS 94	Present	Medium brown	White	Scored	Ellipsoid	Slight
5.	ICS 210	Present	Medium brown	White	Scored	Ellipsoid	Prominent
6.	URAMBU LOCAL	Present	Reddish	Light brown	Painted	Ellipsoid	Prominent
7.	JC 26	Present	Medium brown	White	Cracked	Ellipsoid	Prominent
8.	GPRG 412	Present	Light brown	White	Cracked	Ellipsoid	Prominent
9.	GP 62	Present	Medium brown	White	Cracked	Ellipsoid	Slight
10.	MI 207	Present	Medium brown	White	Scored	Ellipsoid	Slight
11.	RG 3477	Present	Dark brown	White	Cracked	Ellipsoid	Prominent
12.	RG 111	Present	Medium brown	White	Scored	Ellipsoid	Slight
13.	RG 109	Present	Medium brown	White	Cracked	Ellipsoid	Slight
14.	RG 2661	Present	Dark brown	White	Scored	Round	Prominent
15.	ICS 205	Present	Dark brown	White	Cracked	Ellipsoid	Prominent
16.	TNPT 1	Present	Medium brown	White	Scored	Ellipsoid	Prominent
17.	CO 1	Present	White	Medium brown	Scored	Round	Prominent
18.	GPSKI 332	Present	Medium brown	White	Scored	Ellipsoid	Slight
19.	GPSKI 338	Present	Medium brown	White	Cracked	Ellipsoid	Prominent
20.	GPJI 334	Present	Medium brown	White	Cracked	Ellipsoid	Prominent
21.	GP 625	Present	Medium brown	White	Scored	Round	Slight
22.	GP 432	Present	Medium brown	White	Cracked	Ellipsoid	Slight
23.	GP 415	Present	Medium brown	White	Cracked	Round	Prominent
24.	GP 640	Present	Light brown	White	Scored	Ellipsoid	Slight
25.	GP 101	Present	Medium brown	White	Cracked	Ellipsoid	Prominent
26.	MI 154	Present	Medium brown	White	Cracked	Ellipsoid	Slight
27.	MI 163	Present	Light brown	White	Scored	Ellipsoid	Slight
28.	MI 166	Present	Light brown	White	Scored	Ellipsoid	Slight
29.	MI 205	Present	Medium brown	White	Painted	Ellipsoid	Slight
30.	MI 174	Present	Medium brown	White	Scored	Ellipsoid	Slight
31.	MI 171	Present	Medium brown	White	Cracked	Round	Slight
32.	MI 217	Present	Medium brown	White	Scored	Ellipsoid	Prominent
33.	MI 199	Present	Medium brown	White	Cracked	Ellipsoid	Slight
34.	TNPT 3	Present	Medium brown	White	Cracked	Ellipsoid	Slight
35.	DCS 106 – 1	Present	Medium brown	White	Scored	Ellipsoid	Prominent
36.	MI 206	Present	Medium brown	White	Cracked	Ellipsoid	Slight
37.	ICIRG 2272107	Present	Dark brown	White	Cracked	Round	Prominent
38.	ICIRG 19021914	Present	Reddish	Reddish	Scored	Ellipsoid	Slight
39.	ICIRG 1591	Present	Dark brown	White	Scored	Ellipsoid	Slight
40.	ICIRG 26617517	Present	Dark brown	White	Scored	Ellipsoid	Prominent
41.	ICIRG 34255	Present	Medium brown	White	Cracked	Ellipsoid	Prominent
42.	ICIRG 8981	Present	Dark brown	White	Scored	Ellipsoid	Prominent
43.	ICIRG 261325	Present	Medium brown	White	Scored	Ellipsoid	Slight
44.	MI 231	Present	Medium brown	White	Scored	Ellipsoid	Slight
45.	MOHANUR LOCAL	Present	Dark brown	White	Cracked	Ellipsoid	Prominent
46.	DCS 100	Present	Medium brown	White	Cracked	Ellipsoid	Slight
47.	RG 3744	Present	Dark brown	White	Cracked	Ellipsoid	Prominent
48.	RG 27	Present	Dark brown	White	Cracked	Ellipsoid	Prominent
49.	RG 3798	Present	Medium brown	White	Painted	Ellipsoid	Prominent
50.	MCI 9	Present	Light brown	White	Scored	Ellipsoid	Slight
51.	RG 297	Present	Medium brown	White	Cracked	Ellipsoid	Slight
52.	GPJI 370	Present	Light brown	White	Cracked	Ellipsoid	Slight
53.	GPJI 263	Present	Light brown	White	Scored	Ellipsoid	Slight
54.	KUPANOOR LOCAL	Present	Reddish	White	Cracked	Ellipsoid	Prominent
55.	MI 155	Present	Medium brown	White	Scored	Ellipsoid	Slight
56.	NPT LOCAL	Present	Reddish	White	Scored	Ellipsoid	Prominent
57.	PACHAIMALAI LOCAL	Present	Reddish	White	Scored	Ellipsoid	Slight
58.	ICIRG 668257	Present	Medium brown	White	Cracked	Ellipsoid	Prominent
59.	ICIRG 6625113	Present	Medium brown	White	Scored	Round	Slight
60.	MI 161	Present	Light brown	White	Scored	Ellipsoid	Prominent
61.	GP 95	Present	Medium brown	White	Scored	Ellipsoid	Prominent
62.	GP 537	Present	Medium brown	White	Cracked	Ellipsoid	Slight

PSC - presence of secondary coloration; MSC - main seed coloration; SCS - secondary coloration of seeds;
TSC - type of secondary coloration; SS - seed shape; PDC - caruncle protuberance

to dark. Landraces like Kupanoor local, Pachaimalai local, NPT local and Urambu local were reddish, while the variety CO 1 was white in color with round shape (Fig. 1). Based on mottling observed on the seeds, type of secondary coloration was classified as scored, cracked and painted. Frequency of ellipsoid seed shape was more compared to round. Scored and cracked types were more frequent than painted type. Caruncle was present in all the genotypes with almost equal frequency of slight and prominent types. Brown colored, oval shaped seed with caruncle is the most common morphotype observed across Indian germplasm collections. The results are on par with Anjani (2000); Silva *et al.* (2019); Wahibah *et al.* (2020) who have attempted the characterization of castor accessions. Characterization of genetic resources/germplasm is the key step to gain knowledge on specific traits possessed by genotypic collections (Kaur *et al.*, 2022). In arid regions, castor seeds with rounded form and darker colour are thought to be an adaptation for defense against high radiation (Gomez *et al.*, 2016). Only two lines *viz.*, RG 2661 and ICIRG 2272107 were found to possess round seed shape with dark brown colour. Hence, these two lines

should be given consideration during breeding for special ecological regions. When chewed, seeds with visible mottling on their shells have been related to poisoning (Mouser *et al.*, 2007). Hence, seed mottling trait is significant in understanding the toxic principles present in outer shell of castor. Castor caruncle also called as elaiosome is involved in germination and synthesis of triacylglycerol (TAGs) (Wan *et al.*, 2019). Lines with prominent caruncle will be given importance for identifying genes and loci involved in oil content.

Diversity present among any breeding material would arise either by geographical separation or by different forms of natural evolution. Scatter plot depicted the existence of variability among the late flowering monoecious lines of castor (Fig. 2). For grouping genotypes based on genetic distance between them, optimum number of clusters need to be fixed and it was found from NbClust statistical package using average silhouette width (Fig. 3). Cluster analysis classified the genotypes into eight different clusters (Fig. 5). Intra cluster distance (Table 3) was high in cluster II (2.8162), whereas it was low in cluster VII (1.5525). Less genetic distance within the clusters

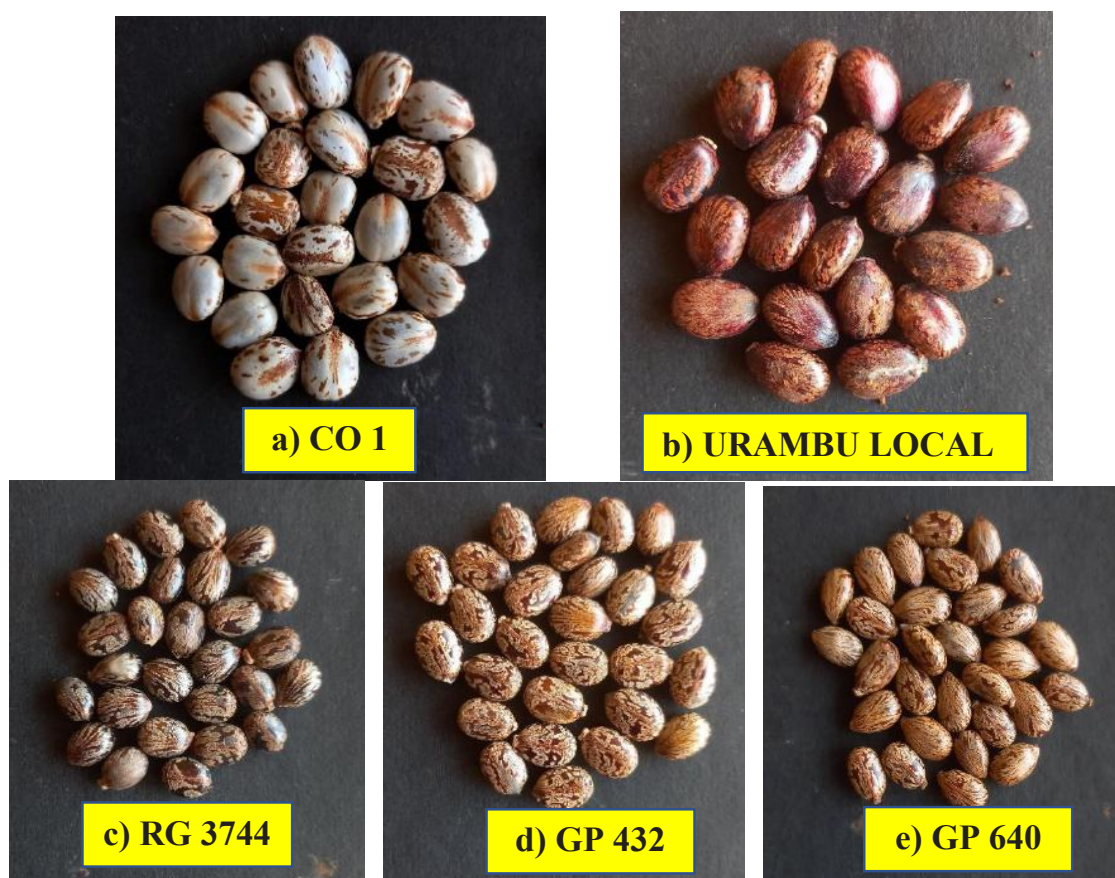


Fig. 1. Morphological variations in colour observed among the seeds of late flowering castor genotypes a) CO 1 - White b) Urambu local - Reddish c) RG 3744 - Dark brown d) GP 432 - Medium brown e) GP 640 - Light brown

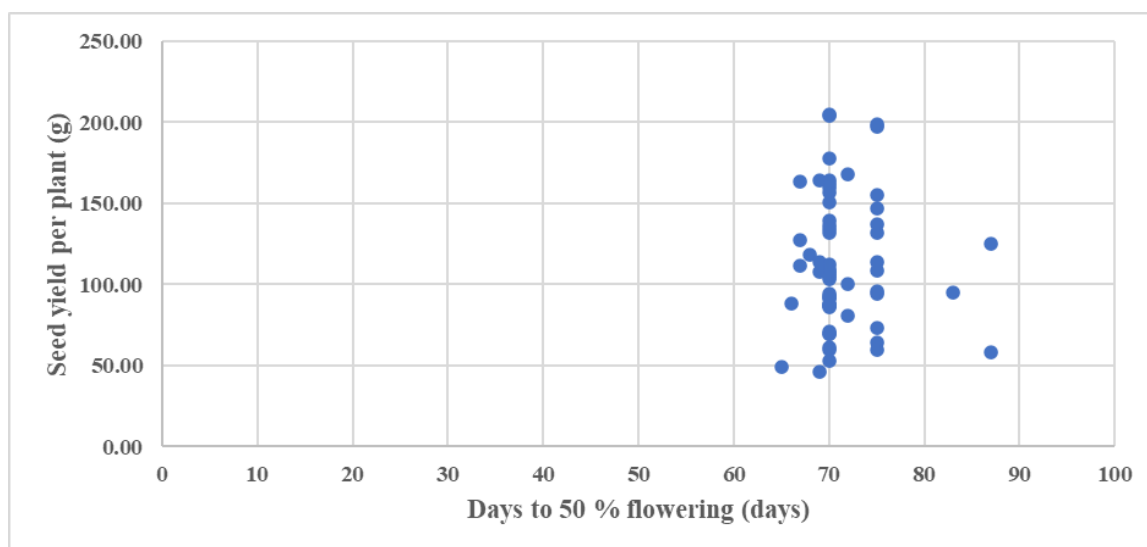


Fig. 2. 2-D scatter plot depicting the days to 50 % flowering (days) and seed yield per plant (g)

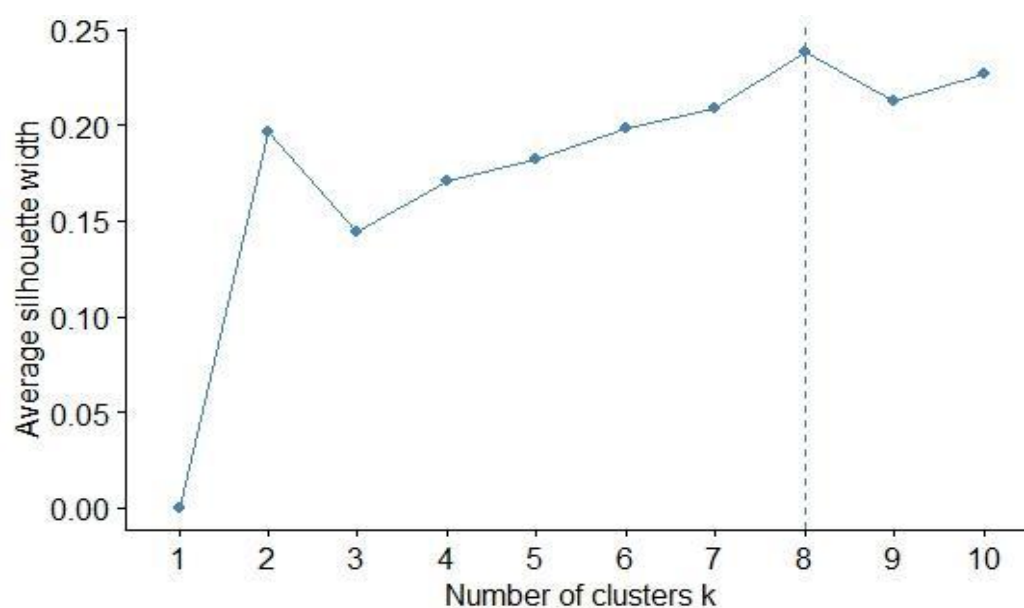


Fig. 3. Optimum number of clusters found using average silhouette width

Table 3. Average inter and intra cluster distance for eight different cluster

Cluster number	C1	C2	C3	C4	C5	C6	C7	C8
C1	2.1478	3.2157	3.0438	3.3311	3.2984	4.9269	4.4945	4.1755
C2		2.8162	3.5717	4.1803	4.7230	4.4144	5.0416	4.5474
C3			2.3503	3.4986	3.0254	4.0284	3.5100	4.7540
C4				1.7579	3.4060	5.0395	3.2961	5.1436
C5					2.3482	4.6755	3.3702	5.1683
C6						2.3314	4.1668	5.6984
C7							1.5525	5.0909
C8								2.3040

indicating that the genotypes present in those clusters share a common genome (Kumar *et al.*, 2022). Cluster II consisted of 11 genotypes, while cluster VII had only four genotypes (Table 4). While considering inter cluster distance, divergence was high between the Clusters VI and VIII (5.6984). Both the clusters possessed only three genotypes each *ie.*, cluster VI had Kupanoor local, GPSKI 338 and DCS 106-1, while cluster VIII had GP 95, MI 161 and GP 537. Effecting hybridization between these genotypes will result in greater hybrid vigour with superior segregants while advancing the generation. Since the number of genotypes were minimum between the distant clusters, any mating design could be followed effectively to reap maximum heterosis. Cluster mean was high for days to 50% flowering in cluster II (85 days) (Table 5). Since this cluster had the highest intra cluster distance with late flowering genotypes, parents could be selected from this cluster to achieve efficient hybridization. 100 seed weight and seed yield per plant was high in cluster VI (44.56 g and 174 g, respectively), while shelling percentage was high in cluster VIII (67.22 %). Cluster mean results also affirmed that parents could be chosen from these two clusters to get novel recombinants. The results are in agreement with the findings of Yamanura

and Mohan (2020) who grouped 33 castor genotypes into eight main clusters; Sadaiah *et al.* (2021) clustered 82 castor germplasm lines into eight clusters and Lavanya and Mukta (2021) classified 72 castor genotypes into nine sub groups using D² analysis.

Seed yield is the most economical trait governed by interplay of many other seed related traits. Hence, assessing the degree of association between these traits would be of immense help in yield improvement. Correlation analysis revealed the presence of high significant positive correlation between seed length and breadth (0.6795) (Table 6 and Fig. 4). L/B ratio was positively influenced by length (0.2509), whereas negative significance was found with breadth (-0.5339). 100 seed weight which is one of the most important yield determining character was positively and significantly correlated with seed length (0.5738) and seed breadth (0.5592). Seed yield per plant was significantly correlated with shelling percentage (0.3092). In order to depict the true picture of relationship between the traits path analysis was done (Table 7) which measures the direct and indirect contribution of various independent trait on the dependent trait yield. Seed length exhibited high positive

Table 4. Cluster size and membership for late flowering monoecious lines of castor

Cluster number	Cluster size	Cluster members
C1	17	GP 432, MI 206, ICIRG 6625113, GPJI 334, MI 163, MI 231, MI 171, MI 155, MI 158, MI 207, MI 201, MI 166, MI 217, RG 111, MCI 9, GP 101, GP 415
C2	11	DCS 100, RG 3744, MI 199, RG 3798, MI 154, GPJI 263, GP 640, ICIRG 1591, MI 159, ICIRG 2272107, ICIRG 8981
C3	11	GPJI 370, Pachaimalai local, ICS 205, TNPT 3, ICIRG 19021914, M3/28-3-1, ICIRG 34255, GPSKI 332, JC 26, GPRG 412, ICIRG 26617517
C4	4	DCS 94, RG 2661, RG 3477, GP 625
C5	9	Urambu local, GP 62, ICIRG 261325, MI 174, RG 109, TNPT 1, NPT local, ICS 210, CO 1
C6	3	Kupanoor local, GPSKI 338, DCS 106-1
C7	4	RG 27, ICIRG 668257, Mohanur local, RG 297
C8	3	GP 95, MI 161, GP 537

Table 5. Cluster mean value for different seed related metric traits of castor

Cluster number	Days to 50% flowering (days)	Seed length (cm)	Seed breadth (cm)	L/B ratio	100 seed weight (g)	Shelling percentage (%)	Seed yield per plant (g)
C1	71.00	1.26	0.66	1.92	30.33	52.47	93.67
C2	85.67	1.07	0.67	1.60	28.90	64.18	92.67
C3	70.00	1.37	0.83	1.65	37.50	62.11	97.44
C4	71.36	0.99	0.57	1.75	25.34	65.86	127.09
C5	69.43	1.16	0.75	1.55	33.24	60.93	170.92
C6	74.25	1.33	0.77	1.73	44.56	66.01	174.00
C7	70.28	1.13	0.72	1.58	28.54	60.65	83.16
C8	71.67	1.30	0.65	1.99	32.67	67.22	153.75

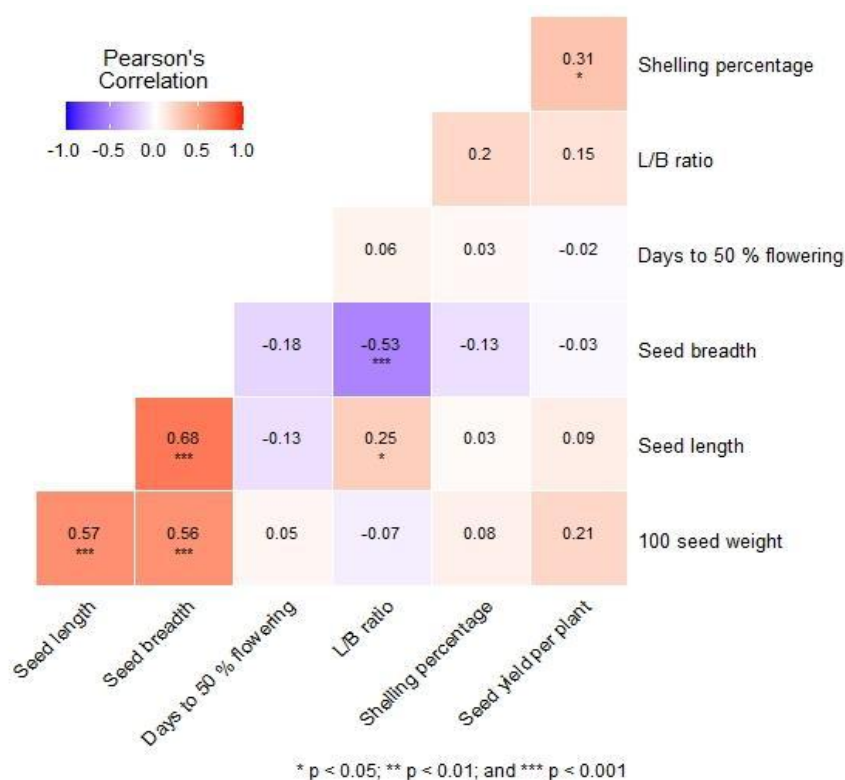


Fig. 4. Heat map generated using the correlation coefficients for six quantitative traits

Table 6. Pearson's correlation coefficient for seed related metric traits of castor

Traits	Days to 50% flowering	Seed length	Seed breadth	L/B ratio	100 seed weight	Shelling percentage	Seed yield per plant
Days to 50 % flowering	1.0000	-0.1347	-0.1794	0.0636	0.0469	0.0346	-0.0235
Seed length		1.0000	0.6795***	0.2509*	0.5738***	0.0254	0.0918
Seed breadth			1.0000	-0.5339***	0.5592***	-0.1342	-0.0343
L/B ratio				1.0000	-0.0724	0.2043	0.1465
100 seed weight					1.0000	0.0764	0.2112
Shelling percentage						1.0000	0.3092*

* p < 0.05; ***p<0.001

Table 7. Path coefficient analysis for monoecious lines of castor using quantitative traits

Traits	Days to 50% flowering	Seed length	Seed breadth	L/B ratio	100 seed weight	Shelling percentage	Correlation coefficient for seed yield per plant
Days to 50 % flowering	-0.0828	-0.0662	0.1278	-0.0244	0.0132	0.0090	-0.0235
Seed length	0.0111	0.4921	-0.4840	-0.0966	0.1625	0.0066	0.0918
Seed breadth	0.0148	0.3343	-0.7123	0.2054	0.1583	-0.0350	-0.0343
L/B ratio	-0.0052	0.1235	0.3803	-0.3849	-0.0205	0.0533	0.1465
100 seed weight	-0.0039	0.2824	-0.3983	0.0279	0.2832	0.0199	0.2112
Shelling percentage	-0.0029	0.0125	0.0956	-0.0786	0.0216	0.2609	0.3092*

Residual effect: 0.1557; * p < 0.05

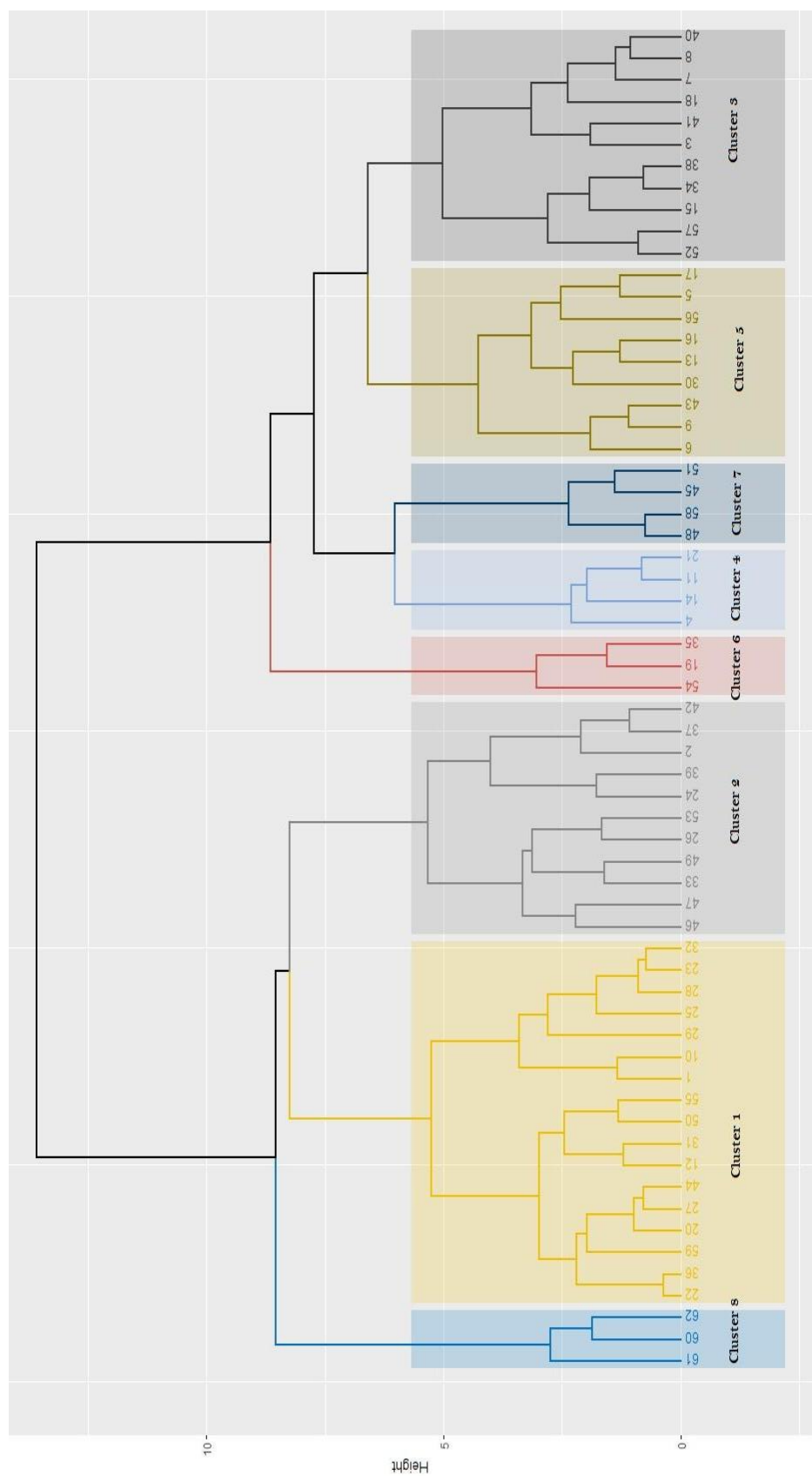


Fig. 5. Dendrogram formed from cluster analysis using late flowering monoecious lines of castor

direct effect (0.4921) and high negative indirect effect through breadth (-0.4840) on seed yield, whereas seed breadth showed high negative direct effect (-0.7123) and high positive indirect effect via length (0.3343). Sadaiah *et al.* (2021) and Anggraeni *et al.* (2022) reported positive significant correlation between 100 seed weight and seed yield per plant which was in agreement with our findings. Therefore, due weightage had to be given to these traits while augmenting yield.

Predominance of brown colour, ellipsoid shape and conspicuous mottling with caruncle was commonly observed among the studied genotypes. These traits will be useful while selecting parents in castor breeding programme aimed at specific trait improvement. Genetic diversity studies revealed two diverse clusters with genotypes Kupanoor local, GPSKI 338, DCS 106-1, GP 95, MI 161 and GP 537. Diversity available within the castor gene pool is low because of its monotypic nature. Genotypes from diverse clusters will be utilized in hybridization programme to reap maximum heterosis. Positive correlation was observed among 100 seed weight with seed length, seed breadth. Seed yield per plant was significantly correlated with shelling percentage. These major yield enhancing traits should be given importance to develop varieties with improved yield.

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