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Identification of potential inbreds using genetic diversity studies in advanced genepool lines of castor (*Ricinus communis* L.)

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ABSTRACT

Castor being an important non-edible oilseed crop requires a constant need for the development of superior hybrids. To achieve the objective, the availability of diverse germplasm is the pre-requisite. One such way to broaden the diversity of germplasm or parental lines is by developing gene pools. The present study was aimed at studying the phenotypic diversity of fifty advanced lines of castor derived from gene pool. Ten agro-morphological traits were evaluated in the genotypes and significant differences were found for all the traits. The genotypes were grouped into ten clusters in D^2 clustering method due to high heterogeneity. The cluster I was the largest with 29 genotypes and the clusters V, VI, VII, VIII, IX and X had only one genotype each. The genetic distance was highest between the clusters III and X. The genetic divergence within the clusters was maximum for cluster IV. The highest cluster means for the characters understudy were distributed across different clusters. The genetic diversity was mostly contributed by plant height up to primary raceme and seed yield. The superior-performing lines for different traits were placed at different clusters in D^2 analysis. Thus to generate heterotic hybrids, such diverse parental lines can be utilized in crossing programmes. However, to further improve the genetic resources of castor, genotypes from diverse clusters with high performance of yield attributing traits can be utilized.

Keywords: *Castor, D^2 analysis, gene pool, clusters, divergence.*

Introduction

Castor (*Ricinus communis* L.) is an industrially important non-edible oilseed crop belonging to the family *Euphorbiaceae*. Castor seed contains 45-55% oil which is the major source of the unusual hydroxyl fatty acid *i.e.*, ricinoleic acid which has tremendous industrial significance (1). It is majorly used as an efficient lubricant for high-speed engines and as an ingredient in several by-products *viz.*, soaps, shampoos, cosmetics, ointments, etc. (2).

In castor, many commercially cultivated varieties and parental lines used for hybrid seed production share a common ancestry which makes them vulnerable to the fluctuating climatic conditions as well as to the newly emerging races of pests and pathogens resulting in compromised yield levels (3). This may be because of the presence of monotypic species in the genus *Ricinus* and the high instability of inbreds especially pistillate lines. Thus exploitation of new genetic resources through the collection or development of genepool, multiple crosses etc. becomes necessary. Vast genetic variation can be expected in castor due to its highly cross-pollinated nature and therefore it is important to characterize the germplasm or breeding lines based on genetic diversity (4). The mahalanobis D^2 statistic is a useful tool to calculate the degree of genetic divergence and for quantitative assessment of the association based on the generalized distance (5).

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It is used to make a quantitative evaluation of genetic divergence. D^2 analysisis a useful tool for plant breeders to categorize genotypes into distinct groups based on genetic divergence between them. It can also be used to analyze the relative contribution of different component traits to total divergence at both the intra- and inter-cluster levels, as well as quantifying the degree of divergence between biological populations at the genotypic level.

Considering the importance of need for diverse genetic base for castor improvement, the present investigation was taken up to identify the superior inbreds for hybrid breeding.

Material and Methods

The current study was conducted at Regional Agricultural Research Station, PJTSAU, Palem, Telangana during *kharif*, 2021. The seed material required for the present experiment includes advanced lines (Table 1) collected from castor genepool(50 No.) developed through random mating of elite lines for three seasons in isolation and also two checks *viz.*PCS-262 and DPC-9. All the 50 genepool lines along with two checks were sown on July 8th, 2021 at Regional Agricultural Research Station, Palem located at 16.5154°N latitude and 78.2493°E longitude. The crop was planted with a spacing of 90 cm between the rows and 60 cm between the plants. Each line was planted in a single row of 6m length and both the checks were repeated 5 times in Augmented Randomized Complete Block Design. The crop was grown in rainfed conditions and all the agronomic practices along with plant protection measures were followed in order to raise it successfully as shown. Genetic divergence was calculated by Mahalanobis' D^2statistical method as (6)and the 50 genotypes and 2 checks were categorized according toTocher's method (1952).

The experimental data was analyzed using INDOSTAT software.

Table 1: List of castor genotypes used in the present study

S.NO	GENOTYPES	S.NO	GENOTYPES
C 1	PRAGATHI	26.	M-GP-91
C 2	DPC-9	27.	M-GP-91-1
1.	M-GP-2	28.	M-GP-104
2.	M-GP-5	29.	M-GP-115
3.	M-GP-8	30.	PCS-371
4.	M-GP-12	31.	PCS-372
5.	M-GP-16	32.	PCS-383
6.	M-GP-21	33.	PCS-385
7.	M-GP-26	34.	PCS-386
8.	M-GP-27	35.	PCS-389
9.	M-GP-28	36.	M-GP-114
10.	M-GP-31	37.	M-GP-119
11.	M-GP-32-1	38.	M-GP-127
12.	M-GP-33	39.	M-GP-134
13.	M-GP-37-1	40.	M-GP-135
14.	M-GP-44	41.	M-GP-139
15.	M-GP-48-1	42.	M-GP-140
16.	M-GP-52	43.	M-GP-145
17.	M-GP-63	44.	M-GP-149
18.	M-GP-65	45.	M-GP-156
19.	M-GP-69	46.	M-GP-160
20.	M-GP-75	47.	M-GP-165
21.	M-GP-76	48.	M-GP-172
22.	M-GP-76-1	49.	M-GP-176
23.	M-GP-77	50.	M-GP-243
24.	M-GP-82		
25.	M-GP-83		

Results and Discussion

In the present study, the data of the ten quantitative traits collected from the 50 genotypes and 2 checks was subjected to ANOVA, and the results are presented in Table 2. For all the quantitative traits *viz*., days to 50% flowering, days to maturity, plant height, number of nodes, raceme length, effective raceme length, number of effective racemes per plant, number of capsules per raceme, 100 seed weight and yield per plot the mean sum of squares were significant. Thus, it indicates the presence of a significant extent of variability in the material considered for the study (7).

Grouping of Genotypes into Clusters

Clustering is a method of determining how closely genotypes are related genetically. When compared to genotypes from other clusters, genotypes in the same cluster are regarded to be more closely connected by their origin, ancestry, and genetic makeup (8-10). Clustering is crucial in crop improvement because it allows selection of diverse parents for obtaining highly heterotic hybrids as well as transgressive recombinants in segregating generations when parents are chosen from several distantly related clusters. Tocher's method has been adapted here in the present study to visualize the clustering pattern.It is a clustering technique that groups similar genotypes based on their traits, facilitating the identification of superior lines thereby enhances the breeding efficiency to focus on promising genotypes forrespective traits (11). The primary idea behind cluster creation is to calculate intra and inter-cluster distances, which serve as a selection index for parents of diverse origins (12). The intra-cluster and inter-cluster values are means calculated from cluster element D^2 values. Crossing genotypes in clusters with a significant inter-cluster distance will be a more accurate method for obtaining acceptable results. It can be utilised as a valid selection criterion and for other breeding purposes (13-15).

In the present study of 50 gene pool lines with two checks,up on subjecting to genetic diversity analysis using Mahalanobis D^2 method yielded a total of ten clusters (Table 3.). Among the ten clusters formed, the largest cluster was the cluster I with 29 genotypes. Next is cluster II with eight genotypes followed by clusters III and IVcomprising of 6 and 3 genotypes, respectively. Whereas the clusters V, VI, VII, VIII, IX and X had only one genotype each (Fig.1&2). This indicates that there is great heterogeneity among the studied genepool lines (16). The lowest inter-cluster distance being 98.24 was noted between the clusters V and VI indicating the genotypes from these two groups were genetically not very diverse. Thus crossing the genotypes from these clusters may not result in larger levels of heterotic expression (15)

Intra and inter-cluster distances were calculated utilizing D^2 values from divergence analysis (Table 4). Among the ten clusters formed, cluster IV had the highest intra-cluster distance of 159.82 followed by cluster III with an intra cluster distance of 130.17. The clusters V, VI, VII, VIII, IX and X had zero intra-cluster distance as they are solitary clusters indicating that they contain genotypes with different and unique genetic makeup (14).

Up on observing these solitary clusters, each entry has got one or the other desirable trait *viz*., M-GP-135 (Cluster V) possesses only 1-2 effective spikes under normal spacing conditions. Thus, the line can be utilized as such in high density planting system to avoid multiple harvesting in castor or can be used as one of the parent in the development of mono-spike or shy branching plant types. Similarly the entry, PCS-372 (Cluster X) has more number of capsules in its primary spike itself compared to other entries which can also be considered as desirable under high-density planting system as much of the yield was attained from primary spike itself which is amicable for mechanical harvesting (17).

The entry M-GP-33 (Cluster VII) had a high test weight (100-seed weight) of 34g compared to the other test entries which can be considered as high under rainfed system. This can be exploited by involving the line in the development high seed-weight cultivars.

The solitary cluster X has noted highest inter-cluster distances with cluster III (1565.86), cluster VI (1396.86), cluster V (1333.16) and cluster I (1226.65) indicating that cluster X is highly diverse among others. Cluster III and IV (1293.13) along with cluster I and IV (955.51) have also been noted to have more inter-cluster distance between them. Thus these clusters have more genetic divergence and hence crossing genotypes from these clusters will yield more heterotic expression as well as a greater range of diversity in succeeding segregating populations (14).

Cluster Means for Different Characters

The cluster means values indicate that there are significant differences among the characters (Table 5) understudy. For days to 50% flowering, the cluster mean was highest in cluster VII (59) and lowest in cluster VIII (50). Days to maturity had the highest cluster mean in cluster III (110) and the lowest cluster mean in cluster VIII (97). Cluster VI had the highest plant height (226.8 cm) and the lowest plant height was noted in cluster VIII (67.2 cm). The highest cluster mean for the number of nodes was noted in cluster III (16.73) and it was the lowest in cluster VIII (9.2). In case of raceme length the highest cluster mean was 65.8 cm which was seen in cluster IX and the lowest was 39 cm in cluster V. For effective raceme length the lowest cluster mean was noted in cluster V (39 cm) and the highest was noted in cluster IX (55.6 cm).

The character number of effective racemes had the highest cluster mean in cluster IX (7.5) and the lowest cluster mean was in cluster V (1.4). Cluster mean for the number of capsules was highest in cluster V (114.4) and lowest in cluster III (44.5). The maximum (34 g) and minimum (22 g) cluster means for test weight were observed in clusters VII and cluster VI respectively. Seed yield was the maximum in cluster X (1783.98 g) and was lowest in cluster III (222.84 g).

In the present study it was found that the solitary cluster X (PCS-372) has showed maximum inter-cluster distance with cluster III (1565.86) and cluster I (1226.65). The genotype from cluster X is a highest yielding yet a late maturing type hence it can be crossed with the early maturing genotype M-GP-104 of cluster III and M-GP-26 of cluster I to produce an early maturing an good plant structure containing hybrids, respectively coupled with high yield.

Contribution of individual characters towards divergence

Of all the ten characters studied, six characters have contributed considerably towards the genetic divergence (Table 6). The character that contributed the most to the genetic diversity was seed yield which affected the diversity by 90.72% and it was ranked 1st for 1203 times. The trait plant height ranged 93 times as 1st which contributed 7.01% towards the diversity. Characters that contributed the most towards the genetic divergence show less diversity within the genotypes. These traits are not inherited in a creditable degree to the following generation, and their expression is significantly reduced.

The traits number of capsules and raceme length contributed 1.13% and 0.82% respectively towards the diversity. The lowest contribution of 0.15% to genetic diversity was by the characters' days to 50% flowering and effective raceme length. There was no contribution from the traits da1ys to maturity, number of nodes, number of racemes and 100 seed weight. The traits with maximum contribution *i.e* seed yield and plant height are important while selecting diverse phenotypes. The percentage contributions of each trait towards genetic divergence are detailed in the Table 6 and fig. 3.

Conclusion

The present study has revealed that, the material understudy has good amount of diversity. The superior performing lines *viz*., MGP-12, MGP-16, MGP-27, MGP-28, MGP-33, MGP-44, MGP-52 etc. have happened to be distributed in different clusters in D2 analysis. Thus such diverse and stable lines can be utilized in crossing programmes to generate heterotic hybrids. For further improvement of castor genetic resources, genotypes from diverse clusters with high performance of yield attributing traits can be utilized.

Future scope of the study: The trait specific divergent lines identified in the present study can be utilized in further breeding programme for the improvement of respective traits in castor.

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Conflict of interest: The authors declare that they have no conflict of interest.

Table 2. Analysis of variance for agro-morphological characters

	Df	DFF	DTM	PH	NN	RL	EFL	NR	NC	TW	PY
Block (ignoring Treatments)	4	19.68**	57.60**	544.02*	4.25*	108.42**	93.34**	1.29**	301.17**	30.14**	168334.70*
Treatment (eliminating Blocks)	1	16.18**	21.58*	1354.24**	8.41*	116.17**	47.56**	2.58**	325.94**	10.15*	143359.00*
Checks	1	72.90**	62.50**	244.03	17.16*	219.01**	0.08**	6.24*	334.08**	0.10	714876.10**
Checks+VarvsVar	50	15.04**	20.76*	1376.45**	8.23*	114.11**	48.51**	2.51	325.78**	10.35*	131928.70*
ERROR	4	0.89	2.75	83.11	1.32	6.08	0.04	0.54	8.18	1.10	15506.10
Block (eliminating Check+Var.)	4	4.60	1.14	123.33	0.28	10.66	6.07	0.66	28.75	2.50	18296.00
Entries (ignoring Blocks)	51	17.36**	26.01*	1387.24**	8.72*	123.84**	54.40**	2.63	347.31**	12.32*	155126.80*
Checks	1	72.90**	62.50**	244.03	17.16*	219.01**	0.081**	6.24*	334.08**	0.10	714876.10**
Varieties	49	16.40**	25.51*	1254.39**	8.56*	114.63**	55.42**	1.96	353.88**	11.77*	106090.40*
Checks vs. Varieties	1	6.75	14.08	9039.80**	8.03	480.06**	58.87**	31.62**	38.30	51.25*	1998161.00**
ERROR	4	0.89	2.75	83.11	1.32	6.08	0.04	0.54	8.18	1.10	15506.16

**Significant at 5 % level, **Significant at 1% level*

Table 3. Distribution of 52 Genotypes into 10 Clusters

Clusters	No. of genotypes	Genotypes
Cluster I	29	M-GP-2, M-GP-8, M-GP-26, M-GP-31, M-GP-32-1, M-GP-37-1, M-GP-44, M-GP-48-1, M-GP-63, M-GP-76-1, M-GP-77, M-GP-82, M-GP-83, M-GP-91-1, M-GP-115, PCS-371, PCS-383, PCS-385, M-GP-114, M-GP-127, M-GP-134, M-GP-139, M-GP-140, M-GP-149, M-GP-156, M-GP-160, M-GP-172, M-GP-176, M-GP-243
Cluster II	8	Pragathi, M-GP-21, M-GP-27, M-GP-65, M-GP-69, PCS-386, PCS-389, M-GP-145
Cluster III	6	M-GP-5, M-GP-75, M-GP-76, M-GP-91, M-GP-104, M-GP-119
Cluster IV	3	DPC-9, M-GP-12, M-GP-16
Cluster V	1	M-GP-135
Cluster VI	1	M-GP-165
Cluster VII	1	M-GP-33
Cluster VIII	1	M-GP-52
Cluster IX	1	M-GP-28
Cluster X	1	PCS-372

Table 4. Average intra and inter eucledian cluster distances among the advanced lines of castor

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X
Cluster I	122.90	239.44	363.74	955.51	165.31	231.25	403.84	494.94	641.31	1226.65
Cluster II		107.81	565.59	745.47	1086.51	413.58	201.25	284.71	430.93	1015.14
Cluster III			130.17	1293.13	254.95	208.99	737.80	833.88	979.22	1565.86
Cluster IV				159.82	1062.00	1125.18	561.88	471.97	326.31	292.29
Cluster V					0	98.24	505.91	605.87	749.68	1333.16
Cluster VI						0	570.31	673.96	812.99	1396.86
Cluster VII							0	112.81	244.07	830.93
Cluster VIII								0	155.41	738.48
Cluster IX									0	590.20
Cluster X										0

Table 5. Cluster Means of the Genotypes for Different Phenotypic Traits

	DFF	DTM	PH	NN	RL	EFL	NR	NC	TW	PY
Cluster 1	53.41	102.66	94.58	13.56	53.93	45.32	4.05	54.21	27.38	561.70
Cluster 2	51.38	102.13	80.00	12.88	51.45	44.48	4.85	54.39	31.38	773.16
Cluster 3	58.00	110.00	147.23	16.73	42.07	39.70	2.35	44.50	25.50	222.84
Cluster 4	54.67	100.33	104.13	13.93	65.03	49.97	5.50	85.47	32.67	1508.25
Cluster 5	53.00	99.00	159.40	15.80	39.00	39.00	1.40	114.40	27.00	452.50
Cluster 6	55.00	101.00	226.80	16.00	45.00	43.40	2.40	74.60	22.00	393.89
Cluster 7	59.00	104.00	125.20	13.00	55.80	53.00	4.00	70.20	34.00	954.72
Cluster 8	50.00	97.00	67.20	9.20	47.00	42.80	7.20	56.00	26.00	1048.32
Cluster 9	54.00	101.00	105.00	15.90	65.80	55.60	7.50	53.20	30.00	1197.00
Cluster 10	57.00	98.00	95.60	13.50	57.60	44.40	5.30	112.20	30.00	1783.98

Table 6. Per Cent Contribution of Different Traits towards Genetic Diversity

Source	Times ranked 1st	Contribution
DFF	2	0.15%
DTM	0	0.00%
PH	93	7.00%
NN	0	0.00%
RL	11	0.82%
EFL	2	0.15%
NR	0	0.00%
NC	15	1.13%
TW	0	0.00%
PY	1203	90.40%

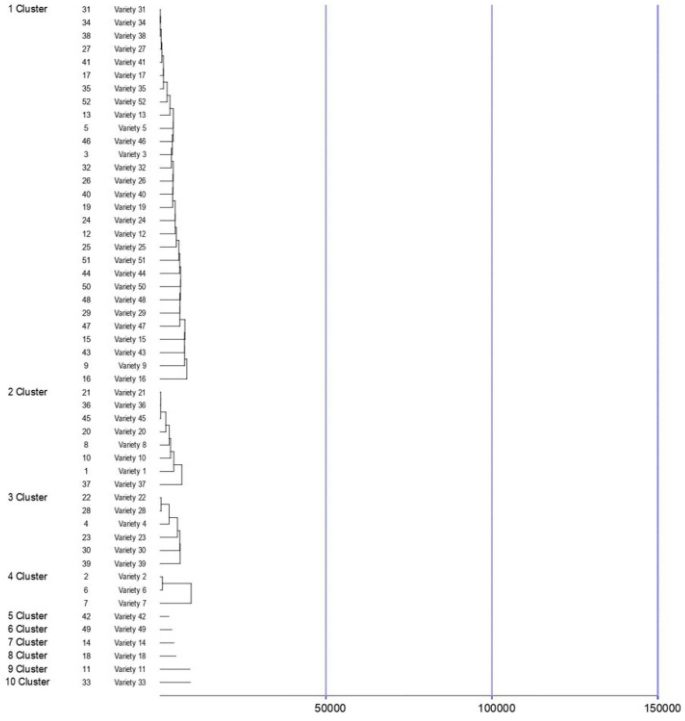

Figure 1. Clustering Pattern of 52 Genotypes by Tocher's Method

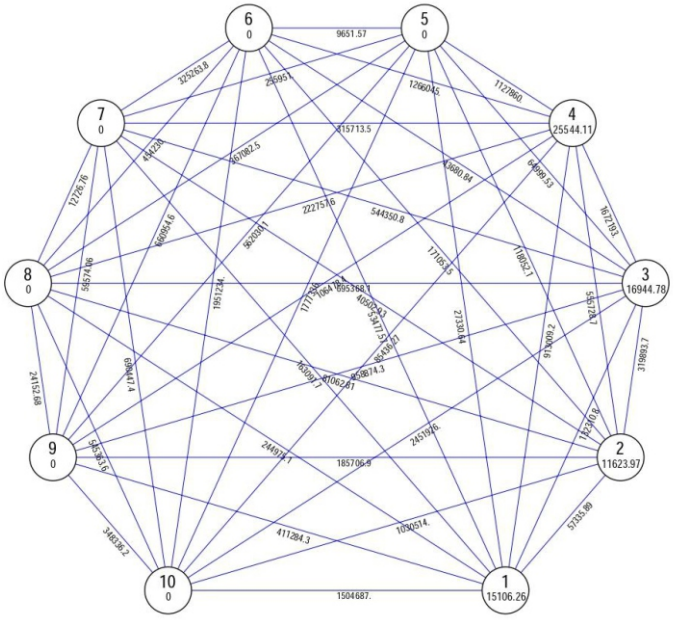

Figure 2. Intra and Inter Cluster Distances

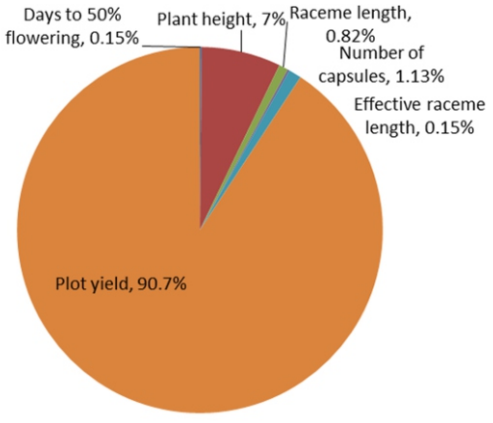

Figure 3. Relative Contributions of Different Characters to Genetic Diversity

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