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Research Note

The role of genetic divergence in determining heterosis in castor (*Ricinus communis* L.)

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Abstract

Combining ability is a useful tool for choosing appropriate parents, while an understanding of the nature of gene action can help develop effective crop improvement initiatives. In order to study if genetic diversity affects hybrid vigour, 50 hybrids were generated by crossing five pistillate lines with ten testers and experimented across two replications during the 2020–21 *kharif*. Genetic divergence was evaluated as Mahalanobis distance among all potential pairs of lines and testers and also in relation to combining ability. Hybrids were classified into four divergent classes using statistics like distance (D^2), overall *gca effects* among parents, overall *sca* effects, and midparent heterotic status across crosses. Genetic diversity studies revealed the that parents to be used in hybridization programmes should be moderately divergent, to derive crosses with high better parent heterosis. Hybrids, *viz.,* MCP-1 × ICS-240, MCP-1 × RG-2787, MCP-1 × RG-3160, YRCP-1 × ICS-258, and DPC-22 × RG-2787, were identified as superior to standard checks for economically important traits. Superior male and female base populations can be created through random mating of lines and testers with high breeding values, such as DPC-22, MCP-1, ICP-30, RG-2787, RG-3160, RG-72, and YRC-1904, respectively.

Keywords: Genetic Divergence, Combining ability, Castor, heterosis

Castor (*Ricinus communis* L.), an oil seed crop of Euphorbiaceae, is possibly one of India's most under-appreciated assets, as castor oil production is vital to Indian agriculture. Castor bean contains 48 *percent* oil, of which 42 *percent* could be extracted. Versatile uses of castor oil after value addition confer the presence of ~85% of a unique hydroxy fatty acid, *i.e.,* ricinoleic acid. In India, castor is cultivated in 0.93 m.ha with a production of 1.98 m.t and a productivity of 2,048 kg/ha (Anonymous, 2023–24). Some of the top hybrids of recent times include ICH-66 (SKP-84 × ICS-164) with a produce of 1560 kg/ha (rainfed) and GCH-9 (SKP-84 × PCS-124) with a produce of 3820 kg/ha (irrigated) (ICAR-IIOR,2022-23). However, the area of cultivation in India and elsewhere is decreasing due to several biotic stresses, namely, grey rot, wilt, leaf-eating caterpillars, capsule borer, and sucking pests (Lavanya *et al.,* 2024). To solve this problem, a high-yielding, drought-resistant cultivar of castor hybrid YRCH 1 suitable for both rainfed and irrigated situations was developed, anticipating that it would boost castor productivity. (Naveena *et al.*, 2021). It is predominantly cross-pollinating (anemophily) due to monoecy; however, self-pollination prevails considerably (Allan *et al.,* 2008). Both genetic and non-genetic variables play a major role in determining the ratio of male and female flowers in a raceme. Heterosis of hybrids over their parents is the most important genetic strategy for increasing productivity of cross-pollinated species in general and castor hybrids in particular. The use of monoecious lines to exploit heterosis in castor is time-consuming, which can be offset by employing pistillate lines to generate commercial hybrids (Gopani *et al.,* 1968). Heterosis derived from crosses involving two unrelated inbreds from contrasting heterotic groups supports greater yield gains. In this context, combining ability studies are essential in evaluating parents and their cross-combinations. It also provides a means of comprehending the nature of gene action that results in the inheritance of target traits. Combining distinct parents in the development of hybrids can result in hybrids with higher levels of heterosis and superior genetic makeup (Gajera *et al.,* 2000). Having a solid understanding of genetic diversity would enable us to identify the diverse genotypes that may be used as parents in a hybridization programme to generate better segregants(Ranjitha *et al.,* 2019). Mahalanobis D^2 is a useful statistic for estimating the extent of genetic divergence based on generalised distance (Mahalanobis, 1936). To this end, a study was undertaken to study the influence of parental diversity on heterosis in Castor.

The present study involved a total of 50 castor hybrids produced from five pistillate lines, *viz.,* YRCP-1, YRCP-2, MCP-1, DPC-22, and ICP-30, and ten testers. RG-72, RG-3798, RG-3160, RG-2787, RG-2722, ICS-240, ICS-253, ICS-258, ICS-234, and YRC-1904. The 50 hybrids were raised in a randomised block design with two replications during *Kharif*, 2020, in the Research Fields of AICRP on Castor, of GKVK, Bangalore, together with their parents and three standard checks, *viz.,* DCH-177, ICH-66, and 48-1. Each entry was raised in a single row measuring 6 m long with a 60 cm plant-to-plant and 90 cm row-to-row distance. Five plants from each genotype were chosen at random, and observations were collected for Days to 50 *percent* flowering, Nodes up to primary spike, Plant height up to base of primary raceme (cm), Total Primary Spike length (cm), Effective primary spike length (cm), Capsules on primary raceme Effective spikes per plant , 100 seed weight (g), Volume weight (g), Seed yield per plant(g) and Oil content (%).

The mean values of all the observations were used for the analysis of variance (ANOVA) as per Panse and Sukhatme (1985) and combining ability analysis as per Kempthorne (1957). Midparent heterosis is highly relevant, as the genetic divergence is calculated as the distance between two parents. Hence, heterosis, as the percent improvement of F_I over the average of the parents for every character, was calculated.

Genetic divergence: The relationship between character-wise parental divergence (Mahalanobis D^2 statistic) and midparent heterosis was determined using linear regression and curvilinear regression of second degree. The analysis of variance for both linear and curvilinear regression was also carried out. A chi-square test was carried out to determine whether the experimental data fit well with either linear regression or a second-degree regression equation using observed and predicted estimates of heterosis. D^2 values were calculated using the sum of squares of the differences between any two genotypes' pairs of corresponding uncorrelated values (Rao, 1952). The mean (m) and standard deviation (s) of the divergence values for 15 parents were determined for parental divergence magnitude. The value between each cross's parents was assigned to a specific divergence class.

Divergent classes are defined as follows:

DC_1: D^2 > or = (m+s)
DC_2: D^2 < (m+s) and > or = m
DC_3: D^2 > or = (m-s) and < m
DC_4: D^2 < (m-s)

Note that DC1 and DC4 are the extremely divergent classes in either direction. For each cross, the divergence class to which the D^2 value between their parents belonged was established. The distribution of crosses with positive heterosis values, the mean for each character, and the number of crosses falling in each divergence class were calculated. Setting a norm for heterosis is essential in order to determine the frequencies of crosses showing heterosis greater than or equal to the norm, as very low positive heterosis values that may not be very significant could get included. The mean heterosis value of these crosses with a positive value of heterosis for that character was considered the norm. The percentage of crosses exhibiting a heterosis value greater than or equal to the norm mean for each character was calculated as well. Also, the highest amount of heterosis observed in every divergence class for every character was noted.The divergence classes were ranked, and in order to come to a final conclusion on the ranking based on positive values of heterosis, mean, and proportion of crosses, a scoring process was adopted. The most desired divergence classes were those with the lowest total score, which had a high average magnitude of heterosis and a high frequency of heterotic crosses. (Arunachalam and Bandyopadhyay, 1984; Mohan Rao, 2000).

ANOVA revealed that for the majority of characters, the variance resulting from lines, testers, and their interaction was significant (**Table 1),** indicating both general combining ability (GCA) and specific combining ability (SCA) variance to be equally essential for the inheritance of all the characters under investigation. Due to the interaction effect, the SCA of certain cross combinations would reflect the GCA of the parents for a particular trait. Thus, choosing parents based on GCA effects is crucial in plant breeding programmes.

A list of parents with their overall combining ability status and hybrids with their overall heterotic status is presented in **Table 2**. The lines MCP-1 and DPC-22, a low and high combiner, resulted in hybrids like MCP-1 × ICS-240 and DPC-22 × RG-2787 that significantly differ from conventional checks (DCH-177, ICH-66, and 48-1) in a favourable direction for many of the traits.

The combination of parents with low overall combining females and high overall combining males has the highest probability of generation of hybrids with high

Table 1. ANOVA for combining ability for seed yield and its attributing traits in castor

Source of Variation	Degrees of freedom	Days to 50% Flowering	Nodes up to primary spikes	Plant height up to primary spike (cm)	Total primary spike length (cm)	Effective primary spike length (cm)	Capsule Primary Spike^{-1}	Effective spikes Plant^{-1}	100SW (g)	Volume weight (g)	Seed yield Plant^{-1} (g)	% Oil
Replicates	1	0.16	58.92 ***	1314.28 **	46.68	66.73	165.07	7.4	1.07	26.20	13.37	4.631
Crosses	49	41.69 ***	5.13 **	828.85***	160.18	136.70 *	319.42 ***	6.56 ***	21.23 ***	11.10	1907.97 ***.	9.94 **
Line Effect	4	56.54	17.45 **	2356.92**	175.65	54.50	970.75 **	18.48 *	34.47	6.43	2328.43	16.41
Tester Effect	9	17.86	6.70	1314.43 *	137.13	147.16	433.72	4.96	41.01 *	6.22	2006.9	21.31 **
Line * Tester Eff.	36	45.99 ***	3.37	537.67 ***	164.22	143.21 *	218.47 ***	5.64 ***	14.809 **	12.84	1836.52 ***	6.37
Error	49	11.63	2.22	156.01	104.17	72.50	61.88	1.91	7.15	8.84	116.53	4.47

*Significance at P = 0.05 level ** Significance at P= 0.01 level ***Significance at P= 0.001 level

Table 2. GCA of parents, SCA, mid parent heterotic status of hybrids in castor

LINES TESTERS	Overall SCA/Heterosis status				
	YRCP-1(L)	**YRCP-2(H)**	**MCP-1(L)**	**DPC-22(H)**	**ICP-30 (H)**
RG-72 (L)	L/H	H/L	H/H	L/H	H/H
RG-3798(H)	H/H	H/H	L/H	L/H	L/H
RG-3160(L)	L/H	H/H	H/L	H/H	H/L
RG-2787(L)	L/H	L/L	L/H	L/L	L/L
RG-2722(L)	L/L	H/L	L/H	H/H	L/L
ICS-240(L)	H/L	HH	H/H	L/L	H/L
ICS-253(L)	H/H	L/L	L/L	H/L	H/H
ICS-258(H)	L/L	L/L	L/H	L/L	H/H
ICS-234(H)	L/L	L/L	H/L	H/H	H/L
YRC-1904(H)	HL	H/L	L/H	L/H	L/L

H: High overall combiner. L: Low overall combiner

Note: H/L or L/H indicates the overall sca status and overall heterotic status respectively.

overall *sca* status. The probability of crosses with a high overall midparent heterotic status belonging to differential parental classes is distributed nearly equally (**Table 3),** indicating that irrespective of the parent's overall combining ability, high heterotic crosses are reflected in all the categories of crosses equally, confirming that parents are showing good dispersion of favourable alleles. The crosses involving poor × poor *GC* parents were found to show low heterosis and high × high combiners, resulting in high heterotic crosses, which are in deviation from the general trend in most of the crops. This is in tune with the findings of Lalitha Reddy *et al.* (2000) on sesamum.

The D^2 statistic was explored to compute pair-wise genetic distances across 15 genotypes and used to find the mean (m), standard deviation (s), and range of genetic divergence across all 50 pairs of parents (**Table 4**). Crosses between genetically dissimilar parents often result in larger heterosis compared to those between closely related parents (Hayes and Johnson, 1939). On the contrary, heterosis is not invariably the result of mating two distinct parents (Cress, 1966). In order to discover the potential limits of parental divergence for the emergence of heterosis, parental divergence was classified as divergent class 1 (DC_1) to divergent class 4 (DC_4) using the mean and standard deviation of all possible D^2 values among the 15 parents (**Table 5**). DC_1 and DC_4 (> m+s or < m-s) represented low and high parent-offspring divergence, respectively, while DC_2 and DC_3 represented a neutral level of parent-offspring divergence (m+s and m-s intervals).

Table 3. Conditional probability of crosses with high levels of overall heterosis and SCA in relation to their parental general combining ability

Category of crosses	No. of crosses	Crosses with high overall sca status	Crosses with high overall heterosis status	Conditional probability of crossings with a high overall sca status.	Conditional probability of crosses with high overall heterosis status
H*H	12	6	8	0.25	0.31
H*L	8	2	5	0.08	0.19
L*H	18	12	7	0.5	0.26
L*L	12	5	6	0.21	0.23
TOTAL	50	24	26		

Table 4. Mean(m), standard deviation (s) and range of genetic divergence values

Statistic	Mean	Standard deviation	Range	
			Highest D^2	Lowest D^2
Value	4.85	0.40	5.28	3.52

Table 5. indicating criteria for classifying genotypes into four divergence classes (DC)

Class	DC_1	DC_2	DC_3	DC_4
Criteria	$D^2 > 5.25$	$4.85 < D^2 < 5.25$	$4.45 < D^2 < 4.85$	$D^2 < 4.45$

Conditional probability results have clearly shown the superiority of DC2, representing moderate parental divergence, indicating that for the occurrence of crosses with heterosis, parents to be used in the hybridization programme should be moderately divergent (**Table 6**). DC_1 (low parental divergence) and DC_4 (extreme parental divergence) had a low chance of generating crosses with significant heterosis. Similar results have been reported by Srivastava and Arunachalam (1977) in *Triticale*.

Overall, high general combiners are not always preferred for producing the best heterotic crosses; nevertheless, deciding whether to use a high or low general combiner from among a specific set of parents can be challenging. It is recommended that, in order to obtain a high frequency of heterotic hybrids, consideration should be given to the genetic distance in addition to combining ability while choosing the parents for hybridization.

Table 6. showing distribution of heterotic crosses in different divergent classes (DC)

Divergence classes	Crosses belonging to that class	Crosses with high overall mid parent heterotic status	Conditional probability of high overall heterotic crosses belonging to a divergence class
DC_1	4	2	0.08
DC_2	29	14	0.56
DC_3	9	6	0.24
DC_4	8	3	0.12

Table 7. showing the comparison of yield traits among six superior crosses

Hybrids	Days to flowering	Grain yield plant^{-1} (g)	Oil content (%)	Grain yield hectare^{-1} (Kg hectare^{-1})	Oil yield hectare^{-1} (Kg hectare^{-1})
MCP-1 × ICS-240	50.00	143.10	50.04	2649.93	1326.00
MCP-1 × RG-2787	56.00	157.50	46.03	2916.59	1341.51
MCP-1 × RG-3160	45.00	170.31	50.06	3153.79	1579.07
YRCP-1 × ICS-258	50.00	184.30	45.37	3412.77	1548.52
DPC-22 × RG-2787	47.00	186.77	46.01	3458.46	1591.00
DCH-177 (Check)	48.50	115.70	46.96	2129.57	1004.99

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