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Assessment of genetic divergence in large seeded genotypes of Groundnut (*Arachis hypogaea* L.) using Mahalanobis D² Statistics

C P Haripriya[1*], P Shanthi[2], K John[3], K Viswanath[4]
[1] Department of Genetics and Plant Breeding, S.V. Agricultural College, Tirupati, Andhra Pradesh, India.
[2] Associate Professor, Department of Genetics and Plant Breeding, S.V. Agricultural College, Tirupati, Andhra Pradesh, India.
[3] Principal Scientist, Department of Genetics and Plant Breeding, Institute of Frontier Technology, Regional Agricultural Research Station, Tirupati, Andhra Pradesh, India.
[4] Principal Scientist, Department of Plant Pathology, Institute of Frontier Technology, Regional Agricultural Research Station, Tirupati, Andhra Pradesh, India
Corresponding Author: C P Haripriya
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Abstract
Genetic divergence is a prerequisite for planning an effective hybridization programme, as crosses between genetically diverse parents are more likely to yield desirable recombinants and superior segregants in the progeny. The present investigation was undertaken to assess the extent of genetic divergence among 32 groundnut (*Arachis hypogaea* L.) genotypes for 15 yield and yield-attributing characters using Mahalanobis D² statistics. Based on Tocher's method, the genotypes were grouped into nine clusters, with cluster I being the largest, comprising 17 genotypes and clusters VI, VII, VIII and IX being monogenotypic. The maximum inter-cluster distance was observed between cluster III and cluster IX (712.64), indicating wide genetic diversity between the genotypes of these clusters, while the minimum inter-cluster distance was recorded between cluster I and cluster IV (136.10). Days to maturity (19.60%), oil content (19.10%) and dry haulms yield plant^{-1} (18.50%) were identified as the major contributors towards total genetic divergence. Based on cluster distances and *per se* performance, genotypes TCGS-2591, TCGS-2674, TCGS-2532, TCGS-2541 and TCGS-2661 were identified as promising divergent parents and specific cross combinations were suggested for exploitation in future groundnut breeding programmes aimed at yield improvement.

Keywords: Groundnut, genetic divergence, Mahalanobis D², Tocher's method, cluster analysis

Introduction
Groundnut (*Arachis hypogaea* L.), often referred to as the 'king of oilseeds', is one of the most important oilseed and food legume crops grown widely in the tropical and subtropical regions of the world. It is a rich source of edible oil (44-56%), protein (25-28%) and other nutrients, making it an important component of global food and nutritional security. Improvement of pod yield, which is a complex quantitative trait governed by several interacting genes and modified considerably by environment, is the principal objective of most groundnut breeding programmes.

Success of any hybridization programme depends largely on the choice of parents and the choice of parents, in turn, depends on the extent of genetic diversity present among them. Genetically diverse parents, when crossed, are more likely to produce a wide spectrum of variability in the segregating generations, providing greater scope for the recovery of desirable transgressive segregants. Mere phenotypic dissimilarity between parents, however, is not always indicative of genetic divergence at the genotypic level; hence, appropriate statistical procedures are required to quantify the actual genetic distance among genotypes.

The Mahalanobis D² statistic (Mahalanobis, 1936) [36] is one of the most powerful and widely used multivariate tools for estimating genetic divergence, as it takes into account the correlated nature of the characters under study. Genotypes are grouped into clusters using Tocher's method (Rao, 1952) [10] based on their D² values, such that genotypes within a cluster are relatively more similar to one another than to genotypes belonging to other clusters. This information can subsequently be used to identify divergent parental combinations that are likely to be exploited for heterosis breeding and for isolating superior recombinants. With this background, the present study was undertaken to assess the magnitude of genetic divergence among 32 groundnut genotypes for yield and its component traits, so as to identify diverse and promising parents for future groundnut improvement programmes.

Materials and Methods
The present investigation comprised 32 groundnut genotypes, which were evaluated in a randomized block design with two replications during the *kharif* 2025 at dryland form of S.V. Agricultural College, Tirupati. Observations were recorded on five randomly selected competitive plants in each replication for 15 quantitative characters, *viz.*, days to 50% flowering, days to maturity, plant height, number of primary branches plant^{-1}, number of mature pods plant^{-1}, hundred pod weight, hundred kernel weight, shelling percentage, sound mature kernel per cent, dry haulms yield plant^{-1}, harvest index, kernel yield plant^{-1}, oil content, protein content and pod yield plant^{-1}.

The genetic divergence among the 32 genotypes was estimated using Mahalanobis D² statistic (Mahalanobis, 1936) [36]. Based on the D² values, the genotypes were grouped into different clusters following Tocher's method as

described by Rao (1952) [10]. The average intra- and inter-cluster D² and D values were computed and a cluster diagram was constructed to depict the genetic relationships among the clusters. Cluster means for all 15 characters were worked out and the relative contribution of each character towards the total genetic divergence was estimated based on the number of times a particular character appeared first in ranking the pairs of genotypes for maximum divergence.

Results and Discussion

Genetic divergence plays a crucial role in the improvement of groundnut by providing opportunities for the identification of diverse parental lines. Knowledge of the magnitude of genetic diversity among genotypes is essential for selecting suitable parents for hybridization, as crosses involving genetically diverse parents are more likely to produce desirable recombinants and superior segregants in subsequent generations. In the present investigation, the genetic diversity among 32 groundnut genotypes was assessed using Mahalanobis D² statistics to quantify the extent of divergence and to identify genetically diverse parents for utilization in hybridization programmes.

Clustering Pattern

The 32 groundnut genotypes were grouped into nine clusters using Tocher's method (Table 1) and (Figure 1). Cluster I was the largest, comprising 17 genotypes, followed by cluster II with five genotypes, while clusters III, IV and V each contained two genotypes. Clusters VI, VII, VIII and IX were monogenotypic, each consisting of a single genotype. The occurrence of both large and monogenotypic clusters indicates the presence of considerable genetic diversity among the genotypes evaluated. Similar clustering patterns in groundnut have earlier been reported by Vivekananda *et al*. (2015) [1], Venkatesh *et al*. (2016) [2], Saritha *et al*. (2019) [3], Saiteja *et al*. (2023) [4], Sukrutha *et al*. (2023) [5] and Sree *et al*. (2024) [6].

Table 1: Distribution of 32 groundnut genotypes into nine clusters based on Tocher's method

Cluster	No. of genotypes	Genotypes included
I	17	TCGS-2595, TCGS-2596, TCGS-2664, TCGS-2592, TCGS-2572, TCGS-2676, TCGS-2672, TCGS-2530, TCGS-2669, TCGS-2589, TCGS-2594, TCGS-2667, TCGS-2666, TCGS-2673,TCGS-2671, TCGS-2534, TCGS-2590
II	5	TCGS-2677, Bheema, TCGS-2593, TCGS-2662, TCGS-2538
III	2	TCGS-2661, TCGS-2541
IV	2	TCGS-2653, TCGS-2529
V	2	TCGS-2539, TCGS-2536
VI	1	TCGS-2674
VII	1	TCGS-2591
VIII	1	TCGS-2532
IX	1	K-7

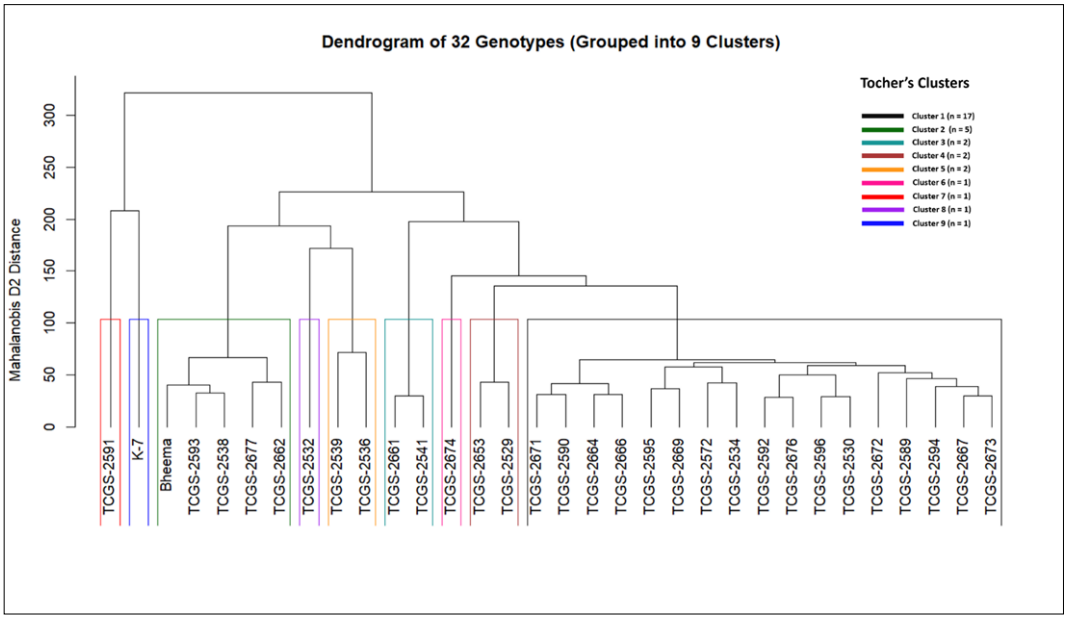

Fig 1: Grouping of 32 genotypes of groundnut into nine clusters

Intra and Inter-cluster Distances

The average intra- and inter-cluster D² values are presented in Table 2 and illustrated in Figure 2. The highest inter-cluster distance was recorded between cluster III and cluster IX (712.64), followed by cluster VII and cluster VIII (559.04), cluster VIII and cluster IX (558.38), cluster III and cluster VII (485.19), cluster VI and cluster IX (425.74), cluster III and cluster V (389.43), cluster IV and cluster IX (372.86), cluster II and cluster VII (358.62), cluster II and cluster III (355.53), cluster I and cluster IX (354.80), cluster V and cluster VII (344.80), cluster IV and cluster VIII (336.33) and cluster III and cluster IV (305.92), in decreasing order of magnitude. The least inter-cluster distance was observed between cluster I and cluster IV (136.10), indicating a close genetic relationship between the genotypes grouped in these clusters. The wide inter-cluster

distances observed among the above combinations indicate substantial genetic divergence, suggesting that crosses involving genotypes from these clusters are likely to produce greater heterosis and a broader spectrum of desirable recombinants in subsequent generations.

Intra-cluster distance was highest in cluster V (111.37), followed by cluster II (98.34), cluster I (96.32), cluster IV (80.31) and cluster III (55.48), indicating relatively greater genetic divergence among the genotypes within these clusters.

Since the inter-cluster distances were consistently higher than the intra-cluster distances, this confirms the presence of substantial genetic diversity among the genotypes studied. Genotypes belonging to clusters III and IX, clusters VII and VIII, clusters VIII and IX, clusters III and VII and clusters VI and IX may therefore be considered as promising parental combinations for hybridization, as crosses between them are likely to generate wide variability and transgressive segregants, facilitating effective selection for desirable traits in groundnut improvement.

Table 2: Average intra- (diagonal) and inter-cluster D^2 distances among nine clusters

Cluster	I	II	III	IV	V	VI	VII	VIII	IX
I	96.32 (9.81)	201.72 (14.20)	179.64 (13.40)	136.10 (11.67)	191.17 (13.83)	138.71 (11.78)	219.53 (14.82)	245.02 (15.65)	354.80 (18.84)
II		98.34 (9.92)	355.53 (18.86)	287.47 (16.95)	175.23 (13.24)	234.28 (15.31)	358.62 (18.94)	232.26 (15.24)	205.58 (14.34)
III			55.48 (7.45)	305.92 (17.19)	389.43 (19.73)	291.19 (17.06)	485.19 (22.03)	280.49 (16.75)	712.64 (26.70)
IV				80.31 (8.96)	188.98 (13.75)	208.80 (14.45)	243.53 (15.61)	336.33 (18.34)	372.86 (19.31)
V					111.37 (10.55)	259.99 (16.22)	344.80 (18.57)	171.91 (13.11)	288.01 (14.42)
VI						0.00 (0.00)	273.71 (16.54)	295.66 (17.19)	425.74 (20.63)
VII							0.00 (0.00)	559.04 (23.64)	208.01 (14.42)
VIII								0.00 (0.00)	558.38 (23.63)
IX									0.00 (0.00)

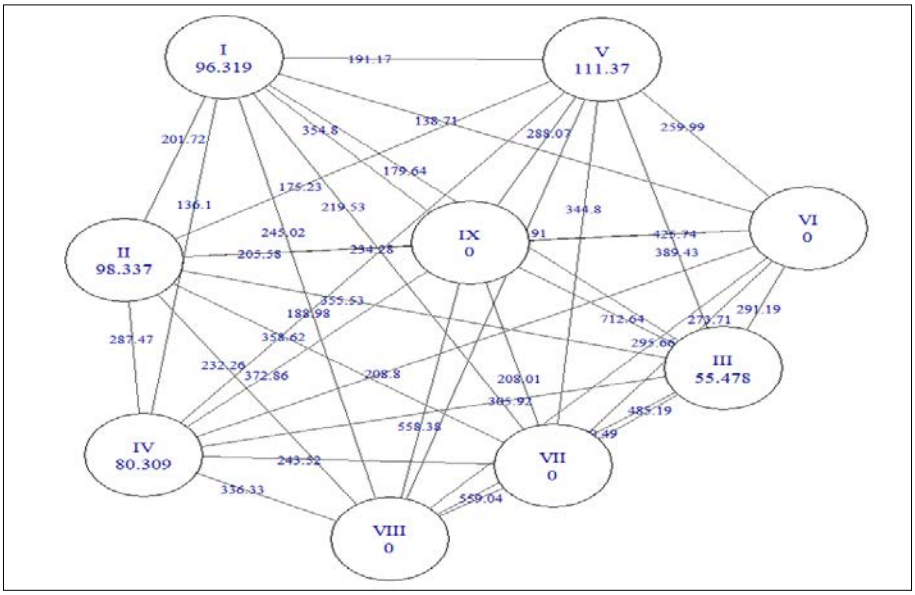

Fig 2: Intra and inter distances (D^2) among nine clusters of groundnut

Cluster Means

Cluster means for yield and yield-related traits are presented in Table 3. Cluster means for days to 50% flowering ranged from 27.00 days (cluster IX) to 34.00 days (cluster VI), with a general mean of 31.94 days; clusters I, VIII and IX showed

Lower mean values, indicating relatively early flowering behaviour. Days to maturity ranged from 104.00 days (cluster VIII) to 114.75 days (clusters V), with a general mean of 111.75 days. Plant height ranged from 33.40 cm (cluster VIII) to 50.00 cm (cluster VI), with a general mean of 45.52 cm.

Table 3: Cluster means for yield and yield attributes in large seeded genotypes of groundnut.

Cluster No.	DFF	DM	PH	NPB	NMP	HPW	HKW	SP	SMK	DHY	HI	KYP	OIL	PRO	PYP
I	31.88	111.62	45.11	6.15	15.52	130.93	46.89	60.12	90.24	27.95	40.88	11.66	48.36	25.03	19.55
II	32.30	114.10	44.90	5.14	14.10	130.83	49.68	66.27	86.83	25.81	40.11	11.62	48.60	25.06	17.49
III	33.75	114.50	47.21	6.75	14.55	127.47	48.90	61.76	90.15	31.01	36.75	11.07	50.07	24.00	17.95
IV	32.00	105.75	49.34	6.75	12.80	138.10	48.89	60.70	84.18	38.00	31.79	10.79	47.56	26.15	17.90
V	32.50	114.75	47.72	5.50	16.95	133.34	46.16	53.85	88.37	33.11	40.60	12.14	47.03	26.61	22.55
VI	34.00	113.00	50.00	6.10	13.90	147.42	51.71	56.80	86.38	44.59	31.27	11.55	47.22	26.04	20.28
VII	32.50	113.50	46.70	5.10	10.60	164.39	48.05	53.43	87.26	27.25	38.59	9.11	48.16	24.86	17.03
VIII	31.50	104.00	33.40	4.80	11.60	128.98	46.88	66.10	89.54	21.07	41.93	10.11	46.90	27.00	15.33
IX	27.00	114.50	45.35	5.50	11.00	118.10	39.41	53.89	87.58	26.95	32.64	7.05	47.96	25.41	13.06
Mean	31.94	111.75	45.52	5.75	13.45	135.50	47.39	59.21	87.83	30.64	37.17	10.57	47.98	25.57	17.90

DFF: Days to 50% flowering, DM: Days to maturity, PH: Plant height, NPB: Number of primary branches plant^{-1}, NMP: Number of mature pods plant^{-1}, HPW: Hundred pod weight, HKW: Hundred kernel weight, SP: Shelling percentage, SMK: Sound mature kernel per cent, DHY: Dry haulms yield plant^{-1}, HI: Harvest index, KYP: Kernel yield plant^{-1}, OIL: Oil content, PRO: Protein content, PYP: Pod yield plant^{-1}

Number of primary branches plant^{-1} ranged from 4.80 (cluster VIII) to 6.75 (clusters III and IV), with a general mean of 5.75. Number of mature pods plant^{-1} varied from 10.60 (cluster VII) to 16.95 (cluster V), with a general mean of 13.45. Hundred pod weight ranged from 118.10 g (cluster IX) to 164.39 g (cluster VII), with a general mean of 135.50 g, while hundred kernel weight ranged from 39.41 g (cluster IX) to 51.71 g (cluster VI), with a general mean of 47.39 g.

Shelling percentage varied from 53.43% (cluster VII) to 66.27% (cluster II), with a general mean of 59.21%. Sound mature kernel per cent ranged from 84.18% (cluster IV) to 90.24% (cluster I), with a general mean of 87.83%. Dry haulms yield plant^{-1} ranged from 21.07 g (cluster VIII) to 44.59 g (cluster VI), with a general mean of 30.64 g and harvest index varied from 31.27% (cluster VI) to 41.93% (cluster VIII), with a general mean of 37.17%.

Kernel yield plant^{-1} ranged from 7.05 g (cluster IX) to 12.14 g (cluster V), with a general mean of 10.57 g. Oil content ranged from 46.90 % (cluster VIII) to 50.07% (cluster III), with a general mean of 47.98% and protein content varied from 24.00% (cluster III) to 27.00% (cluster VIII), with a general mean of 25.57%. Pod yield plant^{-1} ranged from 13.06 g (cluster IX) to 22.55 g (cluster V), with a general mean of 17.90 g and clusters I, III, V and VI recorded higher cluster mean values than the general mean, indicating that genotypes in these clusters may serve as promising sources for pod yield improvement.

Relative Contribution of Characters towards Divergence

The relative contribution of the 15 characters towards total genetic divergence is presented in Table 4 and Figure 3. Days to maturity contributed the highest towards genetic divergence, accounting for 19.60% and ranking first in 97 genotype pairs, followed by oil content (19.10%, ranked first 95 times) and dry haulms yield plant^{-1} (18.50%, ranked first 92 times). These were followed by harvest index (6.20%, 31 times), plant height (5.80%, 29 times), sound mature kernel per cent (5.40%, 27 times) and number of mature pods plant^{-1} (5.30%, 26 times).

The remaining characters, namely number of primary branches plant^{-1} (3.70%), protein content (3.40%), shelling percentage (2.80%), hundred kernel weight (2.80%), hundred pod weight (2.50%), kernel yield plant^{-1} (2.40%), days to 50% flowering (2.00%) and pod yield plant^{-1} (0.50%), contributed comparatively less towards the total genetic divergence among the genotypes.

Table 4: Relative contribution (%) of individual characters towards genetic divergence

Character	Contribution (%)	Times ranked 1st
Days to maturity	19.60	97
Oil content (%)	19.10	95
Dry haulms yield plant^{-1}(g)	18.50	92
Harvest index (%)	6.20	31
Plant height (cm)	5.80	29
Sound mature kernel per cent	5.40	27
Number of mature pods plant^{-1}	5.30	26
Number of primary branches plant^{-1}	3.70	18
Protein content (%)	3.40	17
Shelling percentage	2.80	14
Hundred kernel weight (g)	2.80	14
Hundred pod weight (g)	2.50	12
Kernel yield plant^{-1} (g)	2.40	12
Days to 50% flowering	2.00	10
Pod yield plant^{-1}	0.50	2

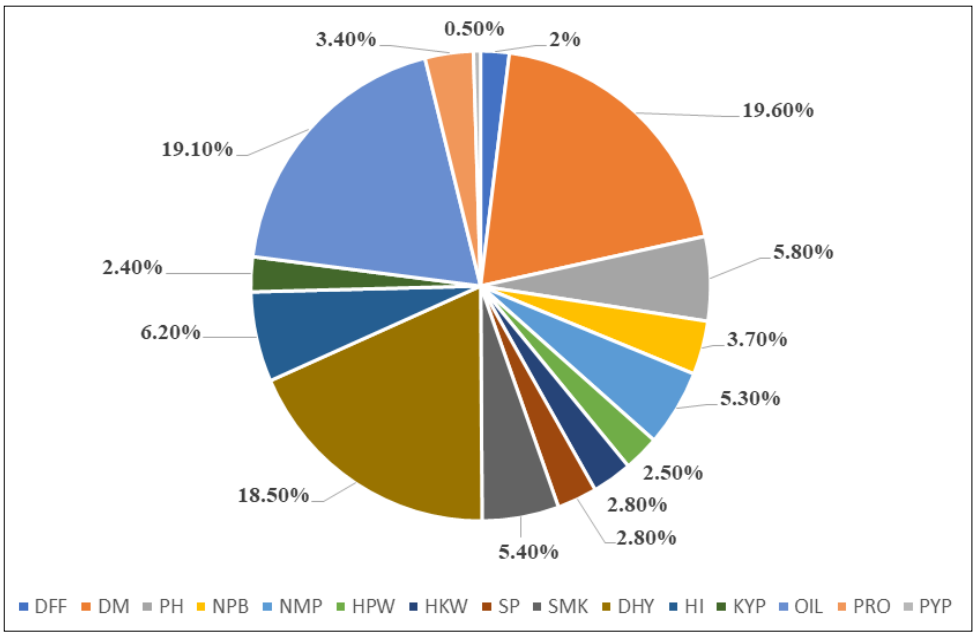

Fig 3: Relative contribution of various characters towards genetic diversity

DFF : Days to 50 % flowering
DM : Days to maturity
PH : Plant height
NPB: Number of primary branches plant^{-1}
NMP: Number of mature pods plant^{-1}
HPW : Hundred pod weight
HKW : Hundred kernel weight
SP : Shelling percentage
SMK%: Sound mature kernel percent
DHY : Dry haulms yield plant^{-1}
HI : Harvest index
KYP : Kernel yield plant^{-1}
OIL : Oil content
PRO : Protein content
PYP : Pod yield plant^{-1}

Days to maturity, oil content and dry haulms yield plant^{-1} thus emerged as the major contributors to the observed genetic diversity among the genotypes and greater emphasis on these traits, together with the superior per se performance of promising genotypes, would be useful in the selection of parents and formulation of effective breeding strategies for groundnut improvement. Similar findings on the maximum contribution of days to maturity towards genetic divergence were reported by Konche deepthi (2021) [7], while the high contribution of dry haulms yield plant^{-1} towards genetic divergence was in agreement with the findings of Sardar *et al* (2017) [8] and Yadav *et al*. (2022) [9].

Identification of Divergent Parents and Promising Cross Combinations

Based on the genetic divergence analysis, the cross combinations K-7 × TCGS-2541, K-7 × TCGS-2661, TCGS-2591 × TCGS-2532, TCGS-2532 × K-7, TCGS-2661 × TCGS-2591, TCGS-2541 × TCGS-2591 and TCGS-2674 × K-7 were identified as promising, since the parental genotypes belonged to highly divergent clusters. These crosses are expected to generate broad genetic variability and can be effectively utilized in groundnut breeding programmes aimed at developing high-yielding and superior varieties.

Further, when genetic divergence was considered together with the *per se* performance of the genotypes, TCGS-2591, TCGS-2674, TCGS-2532, TCGS-2541 and TCGS-2661 emerged as genotypes with superior performance for pod yield and its component traits. These genotypes can therefore be regarded as valuable parental lines and their use in crossing programmes is expected to facilitate the development of high-yielding groundnut genotypes with improved yield-contributing traits.

Conclusion

The present study revealed the existence of substantial genetic divergence among the 32 groundnut genotypes evaluated, as evidenced by their grouping into nine distinct clusters and the wide range of inter-cluster distances observed. Days to maturity, oil content and dry haulms yield plant^{-1} were identified as the principal contributors to the total genetic divergence. Genotypes belonging to the most divergent clusters, particularly clusters III, VII, VIII and IX, along with genotypes TCGS-2591, TCGS-2674, TCGS-2532, TCGS-2541 and TCGS-2661, which also combined good *per se* performance, were identified as promising and genetically diverse parents. Hybridization involving these genotypes is expected to generate wide segregating populations, thereby enhancing the prospects of isolating superior transgressive segregants for pod yield improvement in future groundnut breeding programmes.

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