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Genetic divergence in *makhana* (*Euryale ferox* Salisb.)

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ABSTRACT

Makhana (*Euryale ferox* Salisb.) is an important aquatic cash crop of eastern India. During 2009-10 and 2010-11, an attempt was made to study genetic divergence among 36 *makhana* genotypes using Mahalanobis D^2-statistic at ICAR-RCER, Research Centre for Makhana, Darbhanga (Bihar). Experiments were conducted in field condition in Randomized Block Design with three replications. Data were recorded on 14 morpho-physiological traits, *viz.*, days to germination, days to initiation of flowering, days to 50% flowering, days to 100% flowering, days to initiation of fruit bursting, leaf diameter (cm), fruit diameter (cm), fruits per plant, seeds per fruit, seeds per plant, seed yield per fruit (g), seed yield per plant (g), 100-seed weight (g) and seed diameter (cm). On the basis of D^2 values, these genotypes were grouped into seven clusters. The clustering pattern indicated that cluster I and cluster II were the largest ones accommodating ten genotypes each. While clusters IV and VI were observed the smallest one with single genotype. The highest inter-cluster D^2 values was observed between cluster III and VII (423.20) followed by cluster III and IV (379.69), indicating wider genetic diversity among the groups. The highest intra-cluster D^2 values were observed for the cluster VII (75.90) and the lowest in cluster IV and VII (0.00). Among 14 traits studied, 100-seed weight was observed to have highest contribution (57.30%) towards genetic divergence followed by seeds/ fruit (18.25%) and fruit diameter (17.46%). The results suggested that genotypes BR-5 of cluster III and BR-1 of cluster VII could be the most suitable parents for development of high yielding varieties of *makhana* through hybridization.

Key words: D^2-statistic, *Euryale ferox* Salisb., genetic divergence, inter-cluster distance, seed yield.

INTRODUCTION

Makhana (*Euryale ferox* Salisb.), also known as gorgon nut or fox nut, is one of the most important economic herbs of Nymphaeceae family. This prickly plant with gigantic floating leaves is grown as a cash crop in the low land ecosystem of eastern India comprising parts of Bihar, Assam, West Bengal and Manipur. It is basically grown for its seeds purpose. The commercial value of *makhana* seeds lies in their popped form. Upon popping, the fully matured seeds get converted into white puffs. These starchy white puffs are very nutritious and tasty, and are marketed as a premium dry fruit commodity of *makhana*. Apart from nutritive value, *makhana* seeds are reported to have powerful medicinal properties, and on account of this it is widely used in Aurvedic and Chinese preparations for the treatment of a number of human ailments involving respiratory, circulatory, digestive, excretory and reproductive systems (Kumar *et al*., 2).

To develop high yielding varieties from available genetic materials, the precise information on the genetic divergence is a pre-requisite to the plant breeders in choosing diverse parents for successful hybridization. In this regard, D^2 statistic of Mahalanobis (3) has been extensively used to measure the genetic divergence in several crops. Inter-crossing between more divergent parents is expected to generate a broad spectrum of variability in segregating generations. From breeding point of view, *makhana* is still a new crop. Genetic information on this crop is highly meager and is also not well documented. However, there is one report available on variability studies (Verma *et al.*, 7); but it is based on only a limited number of genotypes and traits, and is much of botanical curiosity. Further, no information is available on genetic divergence in this crop. Hence, an attempt was made to assess the magnitude of genetic divergence in 36 *makhana* genotypes to identify genetically divergent parents for hybridization programme.

MATERIALS AND METHODS

The present investigation was carried out at the Research Farm of ICAR-RCER, Research Centre for Makhana, Darbhanga (Bihar) during the year 2009-10 and 2010-11. The experimental material consisted of 36 *makhana* genotypes. The seeds of these genotypes were collected in year 2009 during a *makhana* exploration trip to various places of Bihar and Manipur states of India. For nursery raising, a shallow field of fertile clay soil was prepared with two deep ploughings followed by puddling. To maintain a level

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of one ft water permanently, an earthen bund of two ft width and 1.5 ft height was made around the field. Seeds of each genotype were broadcast in isolated beds of stagnant watered field in the third week of December month in each consecutive year. Seeds got germinated during the first week of February in the succeeding years. The transplantation of seedlings was made at a spacing of 1.20 cm × 1.25 cm in a well prepared shallow field in the 2nd week of April month. The area under each genotype comprised two rows of 15 m length each. For seedlings transplantation, main field having clay soil was prepared in the same way as nursery block, and during this process a basal dose of N: P: K:: 100:60:40 kg was applied for assuring proper nourishment of *makhana* crop. One ft. stagnating water was maintained in the main field throughout the cropping season.

The observations were recorded on a sample of five randomly selected competitive plants from each plot for 14 morpho-physiological traits, *viz.*, days to germination, days to initiation of flowering, days to 50% flowering, days to 100% flowering, days to initiation of fruit bursting, leaf diameter (cm), fruit diameter (cm), fruits per plant, seeds per fruit, seeds per plant, seed yield per fruit (g), seed yield per plant (g), 100-seed weight (g) and seed diameter (cm). The pooled data were subjected to statistical analysis for different parameters. The D^2 analysis was carried out according to the standard method of Mahalanobis (3). The clustering of genotypes was made by Tocher's method as described by Rao (5).

RESULTS AND DISCUSSION

The pooled analysis of variance revealed significant differences among the genotypes for all the characters studied, indicating existence of sufficient genetic variation in the present set of materials (Table 1). On the basis of D^2 statistic, 36 genotypes were grouped into seven clusters (Table 2). Among these, clusters I and II were observed the largest ones with 10 genotypes each. This was followed by clusters III and VI and cluster V with 5 and 4 genotypes, respectively. The remaining two clusters (IV and VII) were observed to be mono-genotypic. All multi-genotypic clusters included the genotypes of Bihar as well as of Manipur origin, indicating no association between eco-geographical distribution of genotypes and genetic divergence. However, genotypes from the same geographical region were observed to be distributed into different clusters also. This inference is substantiated by the report of Venkateswarlu *et al*. (6) in groundnut crop.

The average intra-and inter-cluster D^2 values and average genetic distance between and within cluster for morpho-physiological traits are presented in Table 3 and depicted in Fig. 1. The maximum intra-cluster D^2 value (75.90) was found in cluster VI, while least intra-cluster divergence (0.00) was observed for clusters IV and VII. As far as the inter-cluster divergence is

Table 1. Analysis of variance for different characters in *makhana* (pooled data).

Characters	Mean sum of squares					
	Replication (2)	Environment (1)	Interactions (2)	Overall sum (5)	Treatment (35)	Error (175)
Days to germination	0.000	2.240	0.129	0.500	16.133**	0.469
Days to flowering initiation	3.393	3.629	0.143	2.140	34.902**	0.969
Days to 50% flowering	3.351	35.041	1.055	8.771	35.861**	1.159
Days to 100% flowering	1.115	214.004	1.421	43.815	57.979**	5.821
Days to initiation of fruit bursting	5.541	18.962	0.782	6.322	87.885**	3.019
Leaf diameter (cm)	33.449	51.041	4.763	25.493	1345.395**	21.977
Fruit diameter (cm)	0.093	0.011	0.105	0.082	3.839**	0.010
Fruits/plant	0.032	0.041	0.980	0.293	13.620**	0.329
Seeds/fruit	3.791	23.337	29.393	17.941	1887.698**	6.330
Seeds/plant	247.351	1344.120	70.018	2875.771	289990.776**	930.462
Seed yield/fruit (g)	20.782	507.226	12.115	114.604	2351.010**	9.846
Seed yield/plant (g)	228.500	402.893	284.796	285.897	402926.213**	2283.502
100-seed weight (g)	1.185	11.115	3.351	4.037	2632.658**	2.516
Seed diameter (cm)	0.000	0.002	0.003	0.002	0.141**	0.001

** Significant at 1 per cent level; Degrees of freedom are shown in parentheses.

Table 2. Clustering pattern of 36 *makhana* genotypes on the basis of genetic divergence.

Cluster No.	No. of genotype(s)	Genotype(s) included	Source
I	10	BR-8, MN-23, MN-16, MN-9, MN-20, MN-17, MN-5, MN-12, BR-10, BR-4	Bihar, Manipur
II	10	MN-7, MN-18, BR-12, MN-19, BR-2, BR-3, BR-9, MN-21, BR-7, MN-10	Bihar, Manipur
III	5	MN-8, MN-22, BR-5, MN-15, MN-2	Bihar, Manipur
IV	1	MN-24	Manipur
V	4	BR-6, MN-3, MN-6, MN-13	Bihar, Manipur
VI	5	MN-1, MN-14, MN-11, MN-13, BR-11	Bihar, Manipur
VII	1	BR-1	Bihar

Table 3. Average intra- (bold) and inter-cluster D^2 and D values among 36 *makhana* genotypes.

Cluster	I	II	III	IV	V	VI	VII
I	**33.46 (5.78)**	72.5 (8.51)	241.54 (15.54)	62.55 (7.90)	129.37 (11.37)	117.99 (10.86)	51.66 (7.18)
II		**35.70 (5.97)**	112.08 (10.58)	52.79 (7.26)	217.61 (14.75)	85.12 (9.22)	155.69 (12.47)
III			**40.47 (6.36)**	214.88 (14.65)	379.69 (19.48)	115.29 (10.73)	423.20 (20.57)
IV				0.00 (0.00)	217.29 (14.74)	163.86 (12.80)	87.52 (9.35)
V					**46.52 (6.82)**	209.57 (14.47)	137.18 (11.71)
VI						**75.90 (8.71)**	233.86 (15.29)
VII							**0.00 (0.00)**

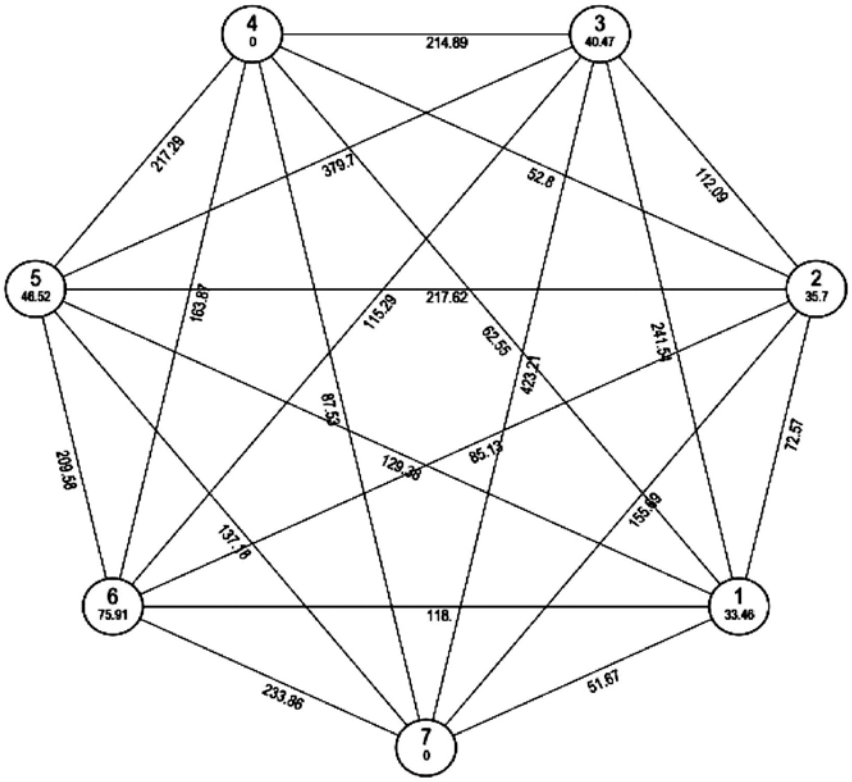

Fig. 1. Statistical distances expressed as D^2 values.

concerned, clusters III and VII were observed to be highly divergent, as indicated by maximum values of D^2 (423.20) and average distance (20.57) between them. The least inter-cluster divergence was recorded between clusters I and VII as indicated by lowest D^2 value (51.66) and average distance (7.18). On the basis of inter-cluster distance, clusters III and VII were identified as the most divergent clusters in the present materials of *makhana* crop. The minimum inter-cluster average D^2 value (51.66) was found between the clusters I and VII followed by 52.79 between clusters II and IV. It revealed that genotypes of these clusters were relatively close to each other as compared to genotypes grouped in other clusters. Such analysis was meant to avoid selection of parents from genetically homogenous clusters in order to maintain a relatively broad genetic base. Similar pattern of genetic divergence has also been reported by Parikh *et al*. in rice (4), and Gaur and Maloo in grass pea (1).

The precise information of relative contribution of component traits to the total genetic divergence helps the breeder to identify desirable genotypes from divergent clusters for fruitful hybridization programme. Among the 14 characters, 100-seed weight contributed the maximum (57.30%) towards genetic divergence followed by seeds per fruit (18.25%) and fruit diameter (17.46%). The contribution of the remaining traits, *viz*., leaf diameter, days to germination, days to flowering initiation, days to 50% flowering, days to 100% flowering, days to initiation of fruit bursting, fruits per plant, seeds per plant, seed yield per fruit and seed yield per plant was not of major significance (Table 4). The highest contribution of 100-seed weight towards genetic divergence was also reported in groundnut crop by Venkateswarlu *et al*. (6).

Cluster means for different characters are presented in Table 5. The cluster V was observed to have the maximum mean values for most of the desirable traits like seed yield per plant (1061.04), seed yield per fruit (89.66), seeds per plant (898.83), seeds per fruit (82.83), fruits per plant (11.41), fruit diameter (7.58) and leaf diameter (151.87). Minimum mean values for number of days to flowering initiation (116.83), days to 50% flowering (119.50), days to 100% flowering (122.83) and days to initiation of fruit bursting (148.33) were noticed in cluster IV. Cluster VII exhibited the highest mean values for 100-seed weight (135.33) and the seed diameter (1.317).

As clusters III and VII were observed to be most divergent, inter-crossing of the genotypes from these clusters could be expected to generate a wide range of variability in the segregating generations for desirable traits. In cluster III, out of total five genotypes, the BR-5 genotype was observed to have highest values of 100-seed weight, seeds/fruit and fruit diameter (most contributing traits towards genetic divergence); therefore, this genotype could be selected for hybridization with the solitary genotype BR-1 from cluster VII.

Table 4. Contribution of different traits towards the genetic divergence in *makhana.*

Trait	Times ranked first	Contribution (%)
Days to germination	8	1.27
Days to flowering initiation	5	0.79
Days to 50% flowering	0	0.00
Days to 100% flowering	1	0.16
Days to initiation of fruit bursting	5	0.79
Leaf dia. (cm)	17	2.70
Fruit dia. (cm)	110	17.46
Fruits/ plant	5	0.79
seeds/ fruit	115	18.25
seeds/ plant	1	0.16
Seed yield/ fruit (g)	1	0.16
Seed yield/ plant (g)	1	0.16
100-seed wt. (g)	361	57.30
Seed dia. (cm)	0	0.00

In summary, based on average inter-cluster distance, relative contribution of component traits in genetic divergence and mean value of individual genotype within divergent clusters, it could be concluded that the genotype BR-5 of cluster III and genotype BR-1 of cluster VII would the most desirable parents for genetic improvement through hybridization programme in *makhana* crop.

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Table 5. Cluster mean for 14 morpho-physiological characters in 36 *makhana* genotypes.

Cluster No.	Days to germination	Days to flowering initiation	Days to 50% flowering	Days to 100% flowering	Days to initiation of fruit bursting	Leaf dia. (cm)	Fruit dia. (cm)	Fruits/ plant	Seeds/ fruit	Seeds/ plant	Seed yield/ fruit (g)	Seed yield/ plant (g)	100-seed weight (g)	Seed dia. (cm)
I	31.283	119.900	123.117	126.083	151.483	132.000	6.613	8.433	45.950	365.133	45.700	361.750	113.933	1.123
II	30.367	118.083	121.150	124.367	148.433	129.067	5.830	9.267	42.883	384.267	35.967	337.333	95.050	0.975
III	30.633	120.533	123.633	126.467	152.300	127.400	5.687	10.067	60.333	577.900	34.900	348.133	65.300	0.810
IV	32.333	116.833	119.500	122.833	148.333	124.833	5.150	11.333	32.167	343.500	32.667	377.500	114.500	1.117
V	31.167	121.000	123.792	129.667	152.958	151.875	7.588	11.417	82.833	898.833	89.667	1061.042	123.333	1.217
VI	30.033	119.867	122.333	125.367	151.100	124.733	7.117	9.833	57.033	537.200	41.467	418.600	83.467	0.887
VII	31.167	120.000	123.000	126.000	153.667	136.167	6.650	9.333	36.833	352.833	43.000	415.333	135.333	1.317

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