



Multivariate Analysis for Genetic Diversity Estimation in Lotus (*Nelumbo nucifera* Gaertn.) Genotypes

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ABSTRACT

Background: Lotus (*Nelumbo nucifera* Gaertn.) is a perennial rhizomatous aquatic plant naturally growing in India from Kashmir to Kanyakumari with enormous phenotypic diversity in shape, size and colour. It serves as a gene pool for breeding new, improved cultivars; nevertheless, plants' natural habitat is disappearing daily due to rapid urbanization driven by population growth. Therefore, the current study was formulated to estimate the genetic diversity for upcoming breeding initiatives and further conservation of available germplasm of lotus.

Methods: In the current study, *in-situ* morphological observations were recorded from thirty-three lotus genotypes between 2022 and 2023, from different locations of Bihar (India). Morphological data on 21 quantitative traits were recorded from selected natural lotus growing site with five replications.

Result: Based on principal component analysis, six principal components were identified, which accounted for 88.97% of total genetic variation. In D² analysis, genotypes were categorized into six different clusters. Cluster II consisted of the maximum number of genotypes (23) followed by cluster I (4). The highest intra-cluster distance was recorded for cluster II (184.40), while the inter-cluster distance was highest between cluster VI and III (5098.07). The study highlights the enormous genetic diversity among the genotypes of lotus and generates information about suitable breeding material(s) for future crop improvement programs. The genotypes from distant clusters may serve as potential genetic material for further selection or a superior heterotic combination for lotus breeding.

Key words: Cluster Analysis, Genetic diversity, Genotypes, Lotus, PCA.

INTRODUCTION

Lotus belongs to the family *Nelumbonaceae*. The genus *Nelumbo* has only two different species in the world viz., *Nelumbo nucifera* Gaertn. (Asian lotus) and *Nelumbo lutea* Willd. (American lotus). In India, lotus is growing naturally from Kashmir to Kanyakumari with enormous phenotypic diversity in shape, size and colour (Goel *et al.*, 2001). It is an aquatic herb with white or red-coloured flowers (Long *et al.*, 2020). The Asian lotus is widely distributed throughout Asia and northern Australia (Li *et al.*, 2014), which used for its ornamental flower, leaves, seed and rhizomes as food and medicine in Asia (Lin *et al.*, 2019; Sharma *et al.*, 2017; Chen *et al.*, 2019). On the basis of the yield potential of flower, seed and rhizome of different genotypes across the globe and their economic uses, *Nelumbo* cultivars are usually categorized into three types: ornamental lotus, rhizome lotus and seed lotus (Li *et al.*, 2020). Similarly based on nature of acclimatization of Asian lotus to diverse climatic conditions, it is further grouped into temperate and tropical types (Zhang and Wang, 2006). In general, the temperate lotus is mainly distributed in East and Northeast Asia and has a short flowering duration and thicker rhizomes. The tropical lotus distributed in Southeast and South Asia has a longer flowering period and thinner rhizomes (Li *et al.*, 2010). The wild-type lotus is the progenitor of the cultivated type and serves as a source of useful genes for the breeding of new, improved cultivars; nevertheless, the natural habitat

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of plants is disappearing daily as a result of the ongoing, enormous growth in the human population. Accurate assessment, preservation and recording of genetic diversity are prerequisites for upcoming crop breeding initiatives. Bihar is blessed with enormous diversity among different aquatic crops including lotus. However, a detailed crop improvement program is lacking in the region due to insufficient systematic research on this crop. Meagre information is available regarding the genetic diversity on the crop among different locations of Bihar and genotypes seem to be poorly characterized. During this study, number of genotypes were evaluated, to review the geographical distribution and genetic divergence of the genotypes. The study aimed for the rapid screening of the genotypes to generate the passport information for genetic diversity in lotus, for future breeding program.

MATERIALS AND METHODS

The experiment was formulated and carried out at Department of Horticulture, School of life Sciences, Sikkim University, Gangtok, Sikkim. An extensive survey was conducted in different provinces of Bihar (India) from June to November of 2022 and 2023. Bihar is situated geographically in the eastern part of India, between latitudes 24°20'10"N and 27°31'15"N and longitudes 83°19'50"E and 88°17'40"E. Ten districts of Bihar namely Nalanda, Begusarai, Vaishali, Madhepura, Saharsha, Muzaffarpur, Supaul, Gopalganj, Darbhanga and Bhagalpur were taken into account as the lotus growing locations for the investigation (Fig 1). *In-situ* morphological observation for twenty-one characters viz., leaf width (cm), leaf length (cm), number of leaf venation, length of petiole (cm), diameter of leaf stalk (mm), length of flower stalk (cm), diameter of flower stalk (mm), number of petals per flower, diameter of flower bud (mm), diameter of flower (cm), weight of flower (g), petal length (cm), petal width (cm), number of stamens per flower, receptacle diameter (mm), number of stigma per flower, number of seeds per receptacle, seed weight (g) per receptacle, rhizome length (cm), rhizome diameter (cm), rhizome weight (g) per 50 cm were recorded from 33 naturally lotus growing location and given codes as BRL and numbered from BRL-01 to BRL-33. Data were randomly recorded from each water reservoir with five replications, each replication is a mean of ten plants. Pooled data of both the years (2022 and 2023) was subjected to multivariate analysis viz., principal component analysis (PCA) and cluster analysis was made using INDOSTAT software (Indo

Statistical Service, New Delhi, India). Standardized data, eigen values and associated eigen vectors were calculated from the covariance matrix (S) between the genotypes.

RESULTS AND DISCUSSION

Principal component analysis

Principal components and their correlation coefficients (Eigen vectors) for twenty-one traits are presented in Table 1. The principal component analysis (PCA) grouped the traits under six principal components (PCs), which accounted for 88.97% of the total variation. The number of stamens per flower (0.308) and seed weight (g) per receptacle (0.340) were correlated positively with PC1, which accounted for 37.543% of total variation. Number of seeds per receptacle and the number of petals per flower were positively correlated and length of petiole and length of flower stalk was correlated negatively with PC2, which accounted for 19.089% of total variation. Rhizome length, rhizome weight, weight of flower and diameter of flower bud were positively correlated with PC3, which accounted for 14.193% of total variation. Diameter of flower stalk and leaf width were correlated negatively and leaf length was correlated positively with PC4, which accounted for 7.798% of total variation. Number of leaf venation was correlated positively with PC5, accounting for 6.159% of total variation. Diameter of flower and petal length was correlated negatively with PC6, which accounted for 4.189% of the total variation. PCA is a useful technique for evaluating genetic variation among accessions which pinpoint the major variables causing variation and highlight the

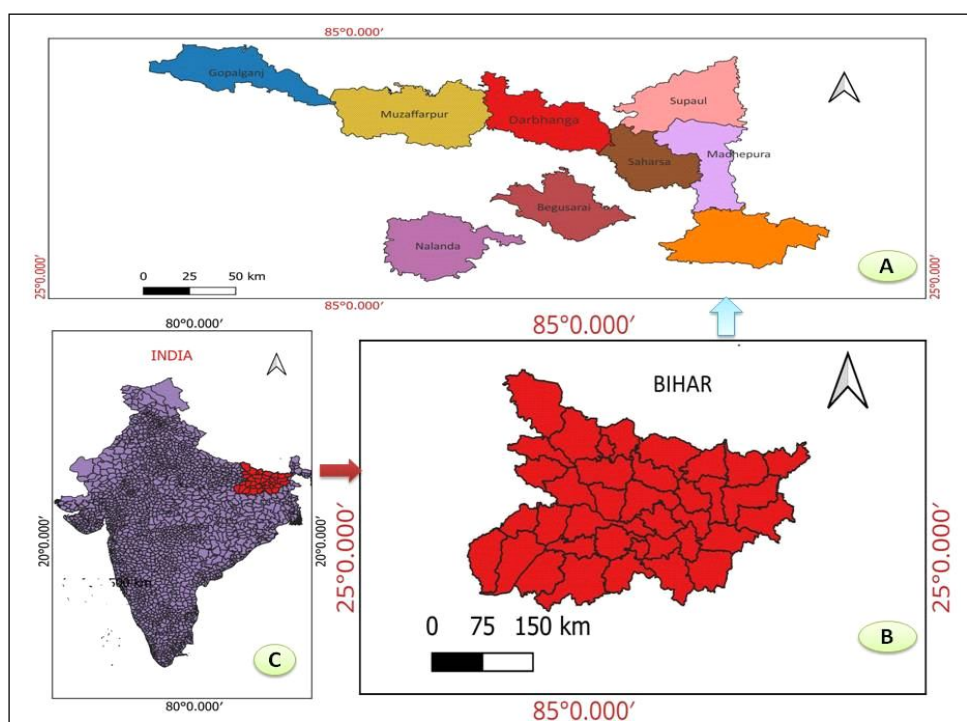


Fig 1: A- Study area of lotus (district wise), B- Study area of lotus (Bihar), C- Study area of lotus (India) as indicated with red colour.

significant variation in genotypes (Verma *et al.*, 2018). The use of principal component analysis (PCA) has proven useful in identifying the genotypes with the most desirable traits for breeding programs in crop plants (Sarkar *et al.*, 2024). Similarly, Guo *et al.* (2010) reported four principal components with 77.33% of total variation in sixty-eight

rhizome lotus (*Nelumbo nucifera* Gaertn) genotypes from China. Six principal components with 84.75% of total variation based on the different physicochemical parameters of the *Garcinia pedunculata* Roxb. accessions were reported from Manipur, India (Hazarika *et al.*, 2023). Su *et al.* (2019) also reported seven principal

Table 1: Principal component analysis explained by six principal components on twenty-one quantitative traits of lotus genotypes.

Quantitative traits	PC1	PC2	PC3	PC4	PC5	PC6
Leaf length (cm)	0.099	0.021	0.377	0.473	0.035	0.177
Leaf width (cm)	0.098	-0.267	-0.057	-0.318	0.173	0.504
Number of leaf venation	-0.192	0.050	0.230	-0.153	0.500	0.088
Length of petiole (cm)	-0.218	-0.357	0.115	0.152	-0.049	-0.015
Diameter of leaf stalk (mm)	-0.139	-0.387	0.086	-0.151	0.146	-0.029
Length of flower stalk (cm)	-0.230	-0.334	0.145	0.184	-0.006	0.010
Diameter of flower stalk (mm)	0.185	-0.019	0.159	-0.502	0.245	0.016
Number of petals per flower	-0.277	0.268	0.102	-0.102	-0.046	0.079
Diameter of flower bud (mm)	0.172	0.246	0.322	-0.126	0.047	-0.230
Diameter of flower (cm)	0.252	0.084	-0.156	-0.132	0.167	-0.531
Weight of flower (g)	-0.274	0.175	0.253	-0.042	0.167	0.001
Petal length (cm)	0.210	-0.178	0.249	0.184	0.249	-0.374
Petal width (cm)	0.211	-0.343	0.140	0.180	0.079	-0.068
Number of stamens per flower	0.308	-0.136	-0.022	0.004	0.205	0.013
Receptacle diameter (mm)	-0.295	0.117	0.133	0.106	0.040	-0.261
Number of stigma per flower	0.252	0.188	0.138	0.139	0.054	0.250
Number of seeds per receptacle	0.258	0.275	0.087	0.175	-0.076	0.207
Seed weight (g) per receptacle	0.340	-0.072	0.005	0.114	0.024	0.137
Rhizome length (cm)	-0.039	-0.022	0.500	-0.157	-0.129	0.024
Rhizome diameter (cm)	0.098	-0.261	0.083	-0.222	-0.521	-0.161
Rhizome weight (g) per 50 cm	0.133	0.029	0.386	-0.246	-0.408	0.027
Eigene Value (Root)	7.884	4.009	2.981	1.638	1.293	0.880
% Var. Exp.	37.543	19.089	14.193	7.798	6.159	4.189
Cum. Var. Exp.	37.543	56.632	70.825	78.622	84.781	88.970

Table 2: Clustering of lotus genotypes.

Clusters	No. of genotypes	Genotypes
Cluster I	04	BRL-01, BRL-08, BRL-21, BRL-22
Cluster II	23	BRL-07, BRL-27, BRL-12, BRL-16, BRL-29, BRL-28, BRL-30, BRL-31, BRL-26, BRL-02, BRL-24, BRL-03, BRL-05, BRL-04, BRL-25, BRL-15, BRL-14, BRL-06, BRL-33, BRL-10, BRL-20, BRL-11, BRL-19, BRL-13,
Cluster III	03	BRL-18, BRL-23
Cluster IV	01	BRL-09
Cluster V	02	BRL-17
Cluster VI	03	BRL-32

Table 3: Intra and inter cluster distance of 33 genotypes of lotus.

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	124.36	665.51	660.10	1039.52	1352.81	4971.24
Cluster II		184.40	563.68	488.60	958.85	4524.67
Cluster III			38.96	1105.48	1565.70	5098.07
Cluster IV				0.00	684.96	2481.97
Cluster V					0.00	2412.98
Cluster VI						0.00

Table 4 (a): Cluster means for 21 characters of 33 genotypes of lotus (Tocher method).

Clusters	Leaf length (cm)	Leaf width (cm)	Number of leaf venation	Length of petiole (cm)	Diameter of leaf stalk (mm)	Length of flower stalk (cm)	Diameter of flower stalk (mm)	Number of petals per flower	Diameter of flower bud (mm)	Diameter of flower (cm)	Weight of flower (g)
Cluster I	42.79	35.12	23.28	154.71	7.25	165.32	6.29	115.48	52.39	10.74	50.46
Cluster II	43.56	37.98	21.90	114.48	7.98	114.88	7.47	16.05	41.27	16.60	21.56
Cluster III	39.30	34.18	21.93	223.86	7.12	230.11	7.20	17.00	39.81	16.96	25.92
Cluster IV	37.47	32.37	22.00	94.10	8.14	98.66	9.30	15.40	57.78	16.25	29.35
Cluster V	63.61	57.13	23.10	91.40	7.82	87.60	9.59	14.50	62.32	18.33	46.55
Cluster VI	56.83	50.61	22.00	133.93	8.67	125.56	9.94	19.00	60.64	18.44	26.43

Table 4 (b): Cluster means for 21 characters of 33 genotypes of lotus (Tocher method).

Clusters	Petal length (cm)	Petal width (cm)	Number of stamens per flower	Receptacle diameter (mm)	Number of stigma per flower	Number of receptacle	Seed weight (g) per Receptacle	Rhizome length (cm)	Rhizome diameter (cm)	Rhizome weight (g) per 50 cm
Cluster I	8.20	3.94	136.38	28.37	15.65	9.23	8.87	120.09	1.53	50.74
Cluster II	10.89	5.87	259.33	30.02	16.83	10.63	11.09	100.70	1.35	54.71
Cluster III	12.32	6.08	271.08	29.74	16.07	9.77	10.29	128.61	1.32	51.03
Cluster IV	11.44	7.62	269.08	30.55	21.20	13.60	14.73	109.80	2.55	141.42
Cluster V	13.54	8.49	335.68	40.93	25.80	14.90	16.33	302.10	1.78	33.31
Cluster VI	11.04	6.27	262.88	28.95	20.40	13.70	15.09	310.28	2.61	266.94

components with a cumulative contribution of 81.86% of total variation under a comprehensive evaluation of 49 waterlily germplasm. Six principal components with a contribution of 84.75 % of total variation were estimated in the bottle gourd (Kumar *et al.*, 2023) and 94.05% of total variation was estimated in the snake gourd (Fathima *et al.*, 2023).

Clustering pattern of the genotypes

Cluster analysis using D² statistics is shown in Table 2. On the basis of D² values, 33 genotypes were categorized into six different clusters. Cluster II was the largest with twenty-three genotypes followed by cluster I with four genotypes. The clusters IV, V and VI possessed only one genotype. The given method of analysis may help to select diverse parents and broaden the local germplasm base for future crop improvement programs. These findings are in close conformity with the results of Guo *et al.* (2010) and Su *et al.* (2019), who reported five clusters based on phenological characteristics of rhizome lotus genotypes and six clusters in waterlily, respectively.

Intra- and inter-cluster distance

Intra- and inter cluster distances are the indicators of genetic diversity between the clusters. Table 3 shows the average intra and inter-cluster distance based on D² analysis. Inter-cluster distances were higher in magnitude than intra-cluster distances in the present investigation, indicating a significant genetic variation among the genotypes under investigation. The intra-cluster distance ranged from 0.00 (cluster IV, V and VI) to 184.40 (cluster II). The minimum intra-cluster distance was zero as only one genotype was present in these clusters. It is clear from table 3 that the highest inter-cluster distance was obtained between cluster III and VI (5098.07) followed by cluster I and VI (4971.24). The diversity between the genotypes might be due to the differences in cultivation history, climatic adaptation and cross-breeding among the genotypes, which causes gene flow between the populations (Ghorbani *et al.*, 2020). The result showed crop improvement programs could employ the distant cluster's genotypes as a viable source to get a broad range of diversity among the segregates and create populations with high yielding transgressive segregates (Singh *et al.*, 2014).

Cluster mean for twenty one characters

Table 4 (a,b) shows the cluster means of different clusters based on twenty-one morphological characters under the study. In clusters I, cluster mean was highest for most of the economic traits viz., number of petals per flower (115.48) and weight of flower (50.46g). Whereas in cluster III, cluster mean was highest for length of petiole (223.86cm) and length of flower stalk (230.11cm). Maximum mean values for rhizome length, rhizome diameter and rhizome weight (g) per 50 cm were recorded in clusters VI [Table 4(b)]. Genotypes with significant mean performance of cluster

and inter-cluster distance could be utilized as potential parents for further breeding programs (Sushil *et al.*, 2023).

CONCLUSION

Principal component analysis and cluster analysis suggest the significant variation for the traits like number of petals per flower, diameter of flower bud, weight of flower, rhizome length, rhizome diameter, number of stamens per flower and seed weight (g) per receptacles from the first three principal components. The highest inter-cluster distance was recorded between clusters III and VI followed by cluster I and VI. This study highlights the considerable level of genetic diversity among lotus genotypes from different locations in Bihar. These genotypes may be subjected to future breeding programs either through direct selection or hybrid breeding.

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Conflict of interest

All the authors of this manuscript declare that they have no conflict of interest.

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