



Multivariate genetic analysis and diversity assessment in lotus (*Nelumbo nucifera*) accessions

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ABSTRACT

The present study was carried out during 2019 to 2022 at the Floriculture Research Station (Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu), Thovalai, Kanyakumari, Tamil Nadu to access the genetic variability in lotus accessions (*Nelumbo nucifera*) collected from 6 diverse locations. The research presents significant insights into the growth and flowering characteristics of these extraordinary aquatic plants and their adaptability to various environmental conditions. The analysis of mean performance showcases noteworthy variations in plant height, leaf dimensions, flowering time and the number of leaves, and flowers/plant across different collection sites, highlighting the profound influence of local environmental conditions on lotus growth. Lotus traits with significant genetic diversity and heritability, presenting opportunities for targeted selective breeding were identified. Principal component analysis uncovers key trait influencers, while cluster analysis categorizes accessions based on similarities, streamlining research and cultivation. Correlation and path analysis unveil relationships among lotus traits, enhancing our understanding of growth factors. D² cluster composition reveals distinct groupings; Cluster I includes KNn1 and KNn5, Cluster II features KNn2, Cluster III comprises KNn6, Cluster IV contains KNn3, and Cluster V houses KNn4. Genetic divergence analysis identifies the number of flowers/plant as the primary driver of genetic diversity, emphasizing its importance for breeding. It underscores the need to consider both genetic and environmental factors in lotus cultivation and highlights the importance of targeted breeding efforts to unlock the full potential of lotus.

Keywords: Correlation, Genetic variability, Genetic diversity, Path analysis, Principal component analysis

Lotus (*Nelumbo nucifera*) is an extraordinary aquatic plant that has long captivated the human imagination with its unique beauty and cultural symbolism. The Nelumbonaceae family, a botanical entity of intriguing uniqueness, is a family by name but monogeneric by nature. Within this family, there exists just one genus, but what an exceptional genus it is, *Nelumbo*. The Nelumbonaceae family encompasses two distinct species, the Asian lotus (*Nelumbo nucifera*); and the American lotus (*Nelumbo lutea*). Its genetic complexity, with a chromosome count of $2n=16$, adds to its allure. Beyond its aesthetic and spiritual value, the lotus is scientifically intriguing, particularly for its genetic diversity. In Asia, notably in India, Thailand, and China, the sacred lotus holds ornamental, religious, medicinal, and edible importance,

enriching its multifaceted presence in the world (Sharma and Goel 2000). The lotus, originating from the murky waters of Asia, has been celebrated for centuries as a symbol of purity, enlightenment, and rebirth. But beneath its serene exterior lies a plant with a rich genetic diversity that has enabled it to adapt and thrive in a variety of environments (Goel *et al.* 2001). Genetic diversity, in the context of the lotus, refers to the wide range of genetic traits and variations found within different lotus populations. Understanding the genetic diversity of the lotus not only sheds light on its evolutionary history but also holds the promise of unlocking its potential for various applications, from agriculture and horticulture to medicine and biotechnology. In this exploration, we will delve into the fascinating world of the lotus and the intricate tapestry of its genetic diversity. By examining the genetic makeup of this remarkable plant, we can gain insights into its resilience, adaptability, and its potential contributions to the fields of science and agriculture. Hence an experiment was planned to evaluate genetic variability and diversity in lotus accessions using principal component, cluster, correlation, and path analyses and identify key traits contributing to genetic diversity in lotus through comprehensive statistical analysis methods.

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MATERIALS AND METHODS

Sampling site and plant material: Present study was carried out during 2019 to 2022 at the Floriculture Research Station (Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu), Thovalai, Kanyakumari, Tamil Nadu. Lotus blooms throughout the year except extreme summer (May) and extreme winter (December) according to the Thovalai season. Six different accessions (KNn-1 Vempathoor - Dark Pink 90%; KNn-2 Pudhugramam - Pink 95%; KNn-3 Thalanthi - Pink 50%; KNn-4 Erachakulam - Pink 25%; KNn-5 Bhemenari - Pink 60%; and KNn-6 Thenthamarikulam - White 75%) were collected from Kaniyakumari, Thiruvattaru, Thuckalay, Nagercoil, Kulasekharam and Pechiparai.

Morphological measurements: The characters observed for this study were six traits, viz. Plant height (m), leaf length (cm), leaf width (cm), days taken from flowering, number of leaves/plant and number of flowers/plant captured upon planting. Six plants from each replication of each treatment were chosen and tagged in order to record observations on different growth and yield features.

Genetic parameters: The statistical analysis was done by adopting the standard procedures (Falconer 1981). The critical difference was worked out at 5% (0.05) probability. In this study, biometrical features were rigorously analyzed using statistical programs SPSS and STAR to derive genetic variability parameters, association studies, and diversity analyses. Genotypic and phenotypic coefficients of variation were calculated from the ANOVA table, as proposed by (Burton 1952), providing measures of genetic diversity (Falconer 1981). The PCV and GCV were categorized into groups based on predefined scales of low, moderate, and high [Mahalanobis (1936), Dewey and Luk (1959), Sivasubramanian and Madhavamenon (1973)]. Heritability was determined following the method in categorized as low (30%), medium (31–60%), and high (>60%) according to Robinson *et al.* (1949). Genetic progress, expressed as a percentage of the mean, was classified as low, moderate, and high (Wright 1921, Johnson *et al.* 1955).

Principal component analysis: Principal component analysis (PCA) through SPSS identified key vegetative and yield parameters contributing to total variation in nerium accessions.

Cluster analysis and character association: Other analyses, including cluster analysis, correlation, path analysis, and genetic divergence were conducted, providing a comprehensive understanding of the complex relationships and diversity within the observed characters.

RESULTS AND DISCUSSION

Mean performance for lotus accessions: The meticulous dissection of mean performance among *Nelumbo nucifera* lotus plants, sourced from disparate geographical niches, unravels a tableau of substantial variations in pivotal attributes, thereby furnishing invaluable glimpses into the idiosyncrasies of their growth and flowering dynamics. Eclipsing its counterparts, Bhemenari emerges as an

arboreal titan, boasting a towering stature of 2.5 m, while the verdant expanse of Vempathoor exhibits a marginally shorter yet respectable elevation of 1.9 m. The arboreal poetry continues with a divergence in leaf dimensions, where Vempathoor reigns supreme, showcasing leaves of unparalleled grandeur, measuring 28.50 cm in length and 31.00 cm in breadth. The temporal ballet of first flowering stages an enthralling performance, wherein Vempathoor takes center stage with an expeditious debut at 38.33 days, juxtaposed against the measured cadence of Erachakulam, unfolding its floral splendor after a protracted gestation of 82.00 days. Thenthamarikulam, in its arboreal symphony, attains pinnacles of distinction, notching up an impressive tally of 16 leaves and 9 flowers/plant, while Pudhugramam and Vempathoor, too, showcase commendable counts, amplifying the botanical diversity. These empirical revelations underscore the profound imprint of local environmental nuances on lotus growth trajectories, unfurling a tapestry of insights that transcend the aesthetic allure of ornamental beauty to the fertile realms of agricultural productivity. The findings serve as compass points for navigating the intricate landscape of harnessing lotus characteristics for multifarious purposes, encapsulating the essence of botanical artistry and horticultural finesse in a quest for both aesthetic and utilitarian gratification.

Collection and survival rate of lotus accessions: The diverse collection of lotus plants from various locations not only showcases a spectrum of flower colours but also highlights significant differences in survival rates, offering crucial insights into the adaptability of *Nelumbo nucifera* in varied environments. The captivating dark pink hue of lotus plants from Vempathoor and the lovely pink colour of those from Pudhugramam add aesthetic elegance, while Thalanthi, Erachakulam, Bhemenari, and Thenthamarikulam exhibit varying shades of pink. Survival rates vary notably, with Pudhugramam boasting an impressive 95%, indicating robust adaptability. Vempathoor follows closely at 90%, while Thalanthi and Erachakulam face challenges with survival rates of 50% and 25%, respectively. Bhemenari and Thenthamarikulam, with pink and white flowers, demonstrate rates of 60% and 75%. These findings highlight the intricate interplay between flower colour and survival rates, suggesting environmental factors influencing lotus thriving. As we navigate lotus cultivation and conservation intricacies, comprehending these variations becomes pivotal for informed decisions and preserving the plant's rich diversity (Wannakraioj 2007).

Genetic variability: Variability parameters estimation for lotus traits unveils a rich genetic diversity, phenotypic variation, heritability, and potential for improvement within various characteristics. Traits like leaf length, leaf breadth, days to first flowering, and number of leaves per plant exhibit high genetic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), indicating substantial genetic diversity and environmental influence. Traits such as plant height and number of flowers/plant show moderate GCV

Table 1 Estimation of variability parameters for lotus accessions

Character	GCV	PCV	Heritability in broad sense h^2 (%)	Genetic advance as percentage of mean (%)
Plant height	12.61	12.99	94.34	24.46
Leaf length	12.28	12.46	97.14	94.43
Leaf breadth	13.80	13.94	98.03	15.80
Days taken for first flowering	26.66	26.71	99.60	81.55
No. of leaves/plant	26.25	27.09	94.63	81.54
No. of flowers/plant	43.96	45.46	93.55	60.60

GCV, Genetic coefficient of variation; PCV, Phenotypic coefficient of variation.

and PCV, balancing genetic and environmental influences. Notably, leaf breadth stands out with low GCV and PCV, indicating stability and limited variability (Table 1). Traits with high heritability and genetic advance, including leaf length and days to first flowering, underscore the significant role of genetics in their variation, offering potential for improvement through selective breeding (Fu *et al.* 2011). Moderately heritable traits like the number of flowers/plant suggest a balanced influence of genetics on flower production, presenting opportunities for moderate improvement. These insights into lotus traits' genetic and environmental influences guide researchers and breeders in enhancing lotus characteristics for ornamental, agricultural, or research purposes by focusing on traits with high genetic diversity and heritability.

Principal component analysis: The revelatory portrayed in Table 2, encapsulating the outcomes of the principal component analysis (PCA) applied to the multifaceted attributes of lotus plants, unfurls an intricate tapestry of distinct patterns and interrelationships threading through these botanical variables. Within this empirical symphony, PC1, commanding a formidable variance of 51.62%, emerges as the maestro, orchestrating a harmonious convergence primarily propelled by the arboreal colossus of plant height, the temporal cadence from first flowering, and the prolificacy encapsulated in the number of flowers per plant. PC1, akin to a botanical virtuoso, weaves a narrative

intricately blending the grandeur of plant stature with the temporal nuances of flowering-related characteristics. PC2 assumes a resplendent role, commanding 33.63% of the variance and unfurling a robust association with the quintessence of flower production, emblematic of a direct linkage to the prolific blooming of petals. PC3, radiating with 12.05% of the variance, casts its botanical spell on the canvas of leaf dimensions and the temporal rhythm of flowering, unveiling an interplay between the verdant expanse and the temporal ballet of floral emergence. PC4, with a modest allocation of 1.95% of the variance, accentuates the temporal dynamics of flowering entwined with the lofty stature of plant height. Meanwhile, PC5 and PC6, in their botanical ballet, contribute minimally to the intricate symphony. The eigenvalues, underscore the commanding authority of PC1, unravelling a substantial expanse of the variance within the botanical milieu. Simultaneously, the cumulative variance percentages ascend as luminous beacons, revealing that the collaborative prowess of PC1 and PC2 captures a staggering 85.24% of the total variance, akin to a botanical diorama painting a vivid panorama of the multifaceted intricacies within the realm of lotus plant attributes.

Cluster analysis: The scrutinized cluster analysis of lotus accessions has culminated in the discernment of three discretely demarcated clusters, each wielding unique botanical signatures. Cluster I, an expansive consortium, embraces a preponderance of accessions, notably KKn1, KKn2, KKn3, and KKn5, aggregating with a total frequency of 4. Cluster II stands as a solitary bastion, characterized solely by the accession KKn4, while the exclusive enclave of Cluster III shelters the singular accession KKn6. An exhaustive examination of the variables nested within each cluster unveils a kaleidoscope of variations spanning plant height, leaf dimensions, flowering chronology, and the numerical abundance of leaves and flowers per plant. Within the hallowed precincts of cluster I, the arboreal magnificence encapsulated in plant height spans a continuum from 1.90–2.70 m, converging at a harmonious mean of 2.17 m and attesting to a paucity of height variability within this botanical congregation, as evidenced by a standard deviation of 0.28. Analogous patterns ripple across diverse variables, including leaf length, leaf breadth, temporal trajectories

Table 2 Principal component analysis eigen values for lotus accessions

Variable	PC1	PC2	PC3	PC4	PC5	PC6
Plant height	0.1685	0.5337	-0.6583	0.4225	0.2734	0.0151
Leaf length	-0.5622	0.0321	-0.1601	-0.0645	-0.0467	0.8068
Leaf breadth	-0.5089	0.0836	-0.4671	-0.4422	-0.2630	-0.5012
Days taken from first flowering	0.5351	0.0621	-0.2311	-0.7505	0.1372	0.2725
No. of leaves/plant	-0.2987	0.5261	0.4429	-0.2412	0.6032	-0.1255
No. of flowers/plant	0.1448	0.6531	0.2707	-0.0199	-0.6864	0.0873
Eigen values	3.0970	2.0171	0.7229	0.1170	0.0460	0
Per cent total variance	51.62	33.63	12.05	1.95	0.77	0
Per cent cumulative variance	51.62	85.24	97.28	99.23	100	100

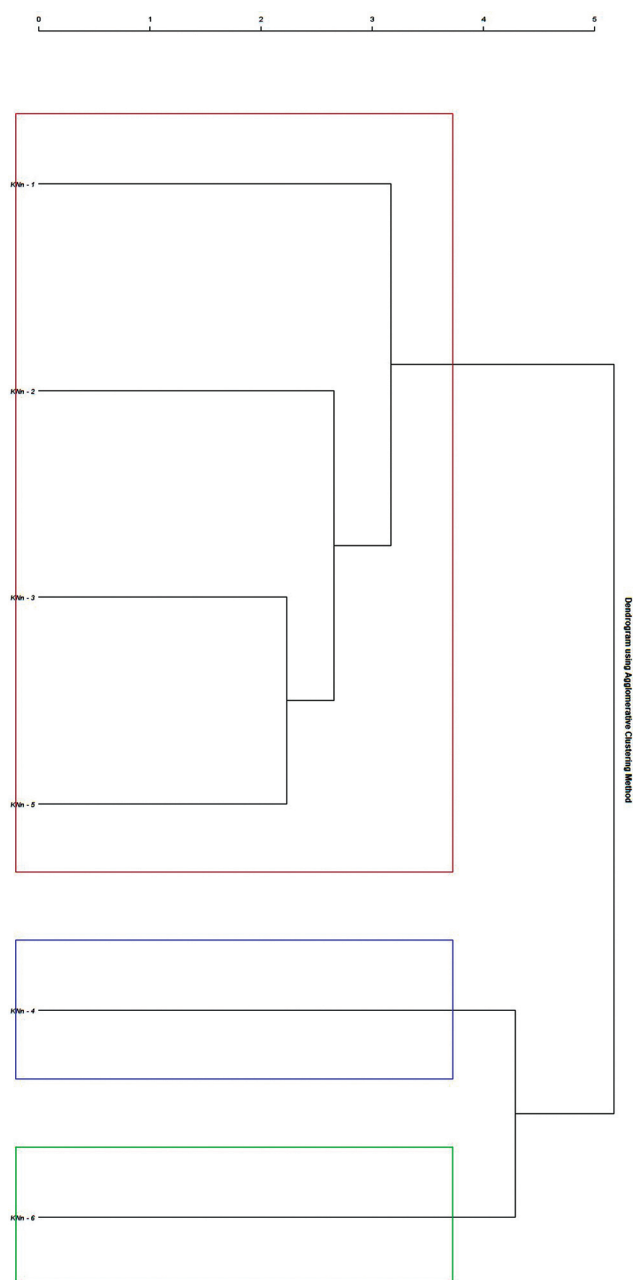


Fig. 1 Cluster analysis dendrogram for lotus accessions.

from first flowering, and the numerical complement of leaves and flowers/plant, resonating with the discernible presence of disparate subgroups meticulously etched within the lotus accessions (Fig. 1). These empirical revelations, shepherded by the erudition of Yanchuang *et al.* (2004), not only offer a profound glimpse into the kaleidoscopic tapestry of diversity inherent in lotus plants but also furnish invaluable compass points for the orchestration of targeted research endeavours and nuanced cultivation strategies tailored to the unique botanical idiosyncrasies encapsulated within each discerned cluster.

Correlation association: The correlation analysis among various lotus plant traits reveals interesting relationships between these characteristics. Plant height exhibits a weak negative correlation with leaf length (-0.186) and a weak positive correlation with days taken from first flowering (0.421), indicating that taller plants tend to take longer to flower. Leaf length is strongly positively correlated with leaf breadth (0.949), implying that as the length of leaves increases, so does their breadth, indicating a proportional growth pattern. However, leaf length has a strong negative correlation with days taken from first flowering (-0.896), suggesting that plants with longer leaves tend to flower earlier. Days taken from first flowering also exhibit a moderate negative correlation with the number of leaves per plant (-0.478), implying that plants taking longer to flower tend to have fewer leaves. Lastly, the number of leaves/plant was positively correlated with the number of flowers/plant (0.627), indicating that plants with more leaves tend to produce more flowers (Table 3 and Fig. 2). These correlations shed light on the interplay between different lotus traits and can inform breeding and cultivation strategies for desired characteristics in lotus plants.

Path analysis: The arboreal colossus, embodied in plant height, emerges as a formidable protagonist, wielding a robust and positively charged direct effect (0.746) on the numerical abundance of flowers/plant, and a judiciously moderate positive direct effect (0.368) on the numerical plethora of leaves/plant. These revelations portend a botanical ballet where taller specimens, in their ascendant

Table 3 Correlation coefficient between various lotus characters and path analysis showing direct and indirect effect of various characters for lotus accessions

Variable	Correlation coefficient between various lotus characters						Path analysis showing direct and indirect effect					
	PH	LL	LB	DFF	NLPP	NFPP	PH	LL	LB	DFF	NLPP	NFPP
PH	1	-0.186	0.021	0.421	0.195	0.64	0.746	0.032	-0.029	-0.146	0.139	0.368
LL		1	0.949**	-0.896*	0.503	-0.24	-0.058	-0.415	-0.504	0.371	0.335	0.564
LB			1	-0.7181	0.415	-0.2	0.041	-0.392	-0.533	0.295	0.204	0.356
DFF				1	-0.478	0.274	0.272	0.384	0.393	-0.401	-0.316	-0.467
NLPP					1	0.627	0.185	-0.247	-0.193	0.225	0.562	-0.325
NFPP						1	0.197	-0.257	0.551	0.456	0.478	0.578

PH, Plant height; LL, Leaf length; LB, Leaf breadth; DFF, Days taken from first flowering; NLPP, Number of leaves/plant; NFPP, Number of flowers/plant.

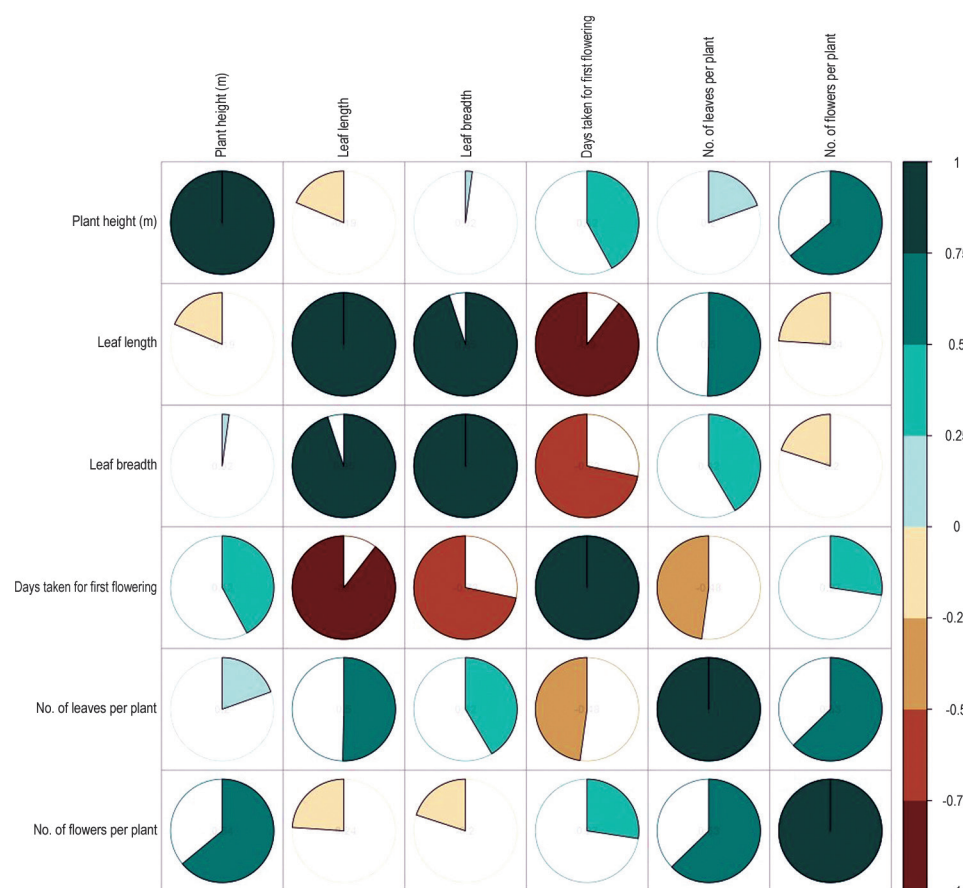


Fig. 2 Correlogram for lotus accessions.

stature, manifest a penchant for direct prodigiousness in both floral and foliar domains. Conversely, the verdant appendages, represented by leaf length and leaf breadth, cast a shadow of negative direct effects on the numerical count of flowers per plant, with coefficients of -0.257 and -0.392, respectively. The temporal rhythm orchestrated by the days taken from first flowering assumes a pivotal role, wielding a potent and inversely charged direct effect (-0.401) on both leaf length and leaf breadth. This botanical minuet unfolds an intricate choreography wherein plants, lingering in the temporal tapestry before flowering, tend to sport shorter and narrower leaves. Furthermore, the temporal ballet extends its influence with a discernibly moderate negative direct effect (-0.467) on the numerical abundance of flowers per plant, portending that a protracted flowering initiation aligns itself with diminished floral prodigality. In the botanical tapestry, the number of leaves per plant emerges as a virtuoso orchestrator, bearing a compelling and positively charged direct effect (0.562) on the numerical surplus of flowers per plant. This botanical revelation intimates that a prolific foliar ensemble precipitates a direct and harmonious augmentation in floral abundance, encapsulating the nuanced interplay of these traits as elucidated (Table 3 and Fig. 3).

Genetic divergence: The composition of D2 clusters for lotus accessions, based on their traits, reveals distinct groupings among the genotypes. Cluster I comprise KNn1

and KNn5, while cluster II was represented by KNn2. KNn6 belongs to cluster III, KNn3 was categorized under cluster IV, and finally, KNn4 is found in cluster V. These clusters highlight the inherent variability in lotus accessions, grouping them based on their trait similarities and differences. This information is valuable for researchers and breeders, as it allows for more targeted and efficient selection and breeding strategies within these distinct clusters to enhance specific traits or characteristics in lotus plants (Yang *et al.* 2013).

The displaying inters and intra-cluster distances for lotus accessions provides valuable insights into the similarity and dissimilarity among clusters. The diagonal elements represent the intra-cluster distances, indicating the average distance between

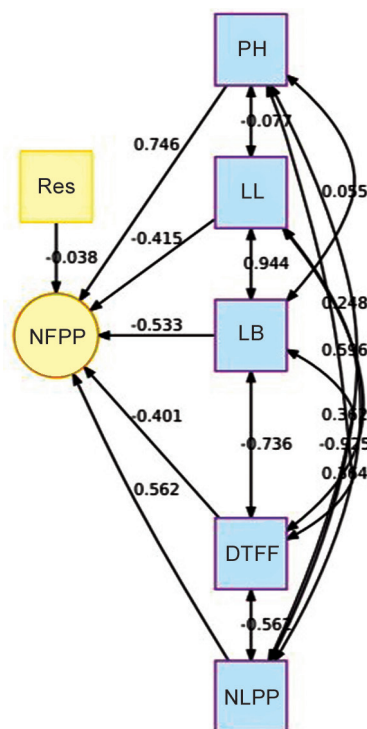


Fig. 3 Path diagram for lotus accessions.

PH, Plant height; LL, Leaf length; LB, Leaf breadth; DTFF, Days taken from first flowering; NLPP, Number of leaves/plant; NFPP, Number of flowers/plant.

genotypes within the same cluster. Notably, cluster 2 (represented by "2") has the smallest intra-cluster distance, indicating high similarity among genotypes within this cluster. The off-diagonal elements signify the inter-cluster distances, revealing the average distance between genotypes from different clusters. Cluster 2, characterized by a distance of 94.311–cluster 1, exhibits relatively closer similarity to cluster 1 compared to other clusters (Chin *et al.* 2017).

The contribution of traits towards genetic divergence in lotus accessions highlights that the number of flowers/plant was the primary driver of genetic divergence, ranking first with a substantial contribution of 100%. This indicates that among the assessed traits, the number of flowers/plant is the most influential factor contributing to the overall genetic diversity and divergence within the lotus accessions under investigation. This information underscores the significance of this particular trait in distinguishing and characterizing different genotypes within the lotus population.

Comprehensive analysis of lotus accessions has provided profound insights into growth, diversity, and genetic characteristics. Variations in plant height, leaf dimensions, and flowering time across diverse sites underscore the influence of local environments on lotus development. Flower colour emerged as a marker for survival rates, revealing adaptability differences. The study identified significant genetic variability among lotus accessions, with KNn1 and KNn5 (cluster I) emerging as superior genotypes, particularly in flower production. These genotypes can be leveraged in lotus breeding programmes to enhance traits such as flower yield and adaptability, aiming to improve ornamental value and agricultural productivity in diverse environmental conditions. Principal component analysis identified plant height, flowering time and the number of flowers as key contributors to genetic diversity in lotus accessions, while cluster analysis grouped the accessions based on these traits, aiding in targeted breeding efforts. The study highlights the number of flowers/plant as the most significant factor in genetic divergence, underscoring its importance in selective breeding. This extensive analysis equips us with knowledge guiding lotus cultivation, conservation and genetic improvement, emphasizing the interplay of environmental and genetic factors in harnessing lotus potential for various purposes.

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