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Research Article

Genetic variability, character association and diversity study in nutmeg (*Myristica fragrans* Houtt)

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Abstract
A panel of 25 nutmeg genotypes were evaluated at the Horticulture Research Station, Pechiparai, Kanyakumari district from 2019 to 2022 for the pattern of genetic divergence and relationship among them based on seven morphological traits by cluster and path analysis. Notable difference was observed for yield characteristics of genotypes, in which the genotype MF-4 was observed to record high plant height, stem girth, leaf length and fruit production. Genetic variability analysis revealed that traits such as stem girth, number of fruits and mace yield/tree were found to possess high genotypic coefficient of variation along with high heritability and genetic advance as percent of mean which implied that these traits had higher variability among the studied genotypes. The 25 genotypes were grouped in to four clusters based on cluster analysis. Path analysis revealed that traits such as number of fruits, single fruit weight and plant height had a favourable direct impact on the mace yield. These traits might be used as selection indicators in future breeding programmes.

Keywords: Path analysis, D^2 analysis, PCV, GCV.

INTRODUCTION

Nutmeg (*Myristica fragrans* Houtt.) is an evergreen tree belonging to the family Myristicaceae, a family of flowering plants indigenous to Asia, Africa, Pacific islands, and America (Kitamura and Poonswad, 2013). It is a fragrant spice, globally cherished as a culinary ingredient. It originated in Indonesia's Moluccas, and it is propagated both sexually and asexually. However, grafting is preferred due to the uneven results in sexual reproduction. Nutmeg genetic diversity manifests through intricate character associations, unveiling relationships between agronomic traits like plant height, stem girth and fruit yield.

Genetic variation is the foundation for any breeding programme. It offers pre-breeding material to the breeder for selection (Shanmugam *et al.,* 2023). Understanding the associations among traits facilitates targeted breeding efforts aimed at optimizing desirable characteristics in this economically significant spice crop. In nutmeg, variability and inter-character associations for fruit weight, fruit number, seed weight, and mace weight have shown that selecting trees with an ideal fruit number and a reasonably excellent seed weight would be beneficial. Identification of diverse parents to produce segregating offspring with the greatest genetic variability and the introgression of desirable genes from diverse germplasm into the existing genetic base are made possible by accurate assessment of genotypes for the proportion and patterns of genetic diversity and variability (Chakravorty *et al.,* 2013). Inheritance of most of the economic traits is complex in nutmeg and mainly involves fruit and tree characteristics. To understand the effect-cause relationships between yield and its constituent parts, it is useful to employ the path coefficient analysis (Vikram *et al.,* 2018). Thus, evaluation of existing variability in the nutmeg genotypes for important economic traits is necessary for successful

crop improvement programmes. Therefore, the goals of this study were to evaluate the level of genetic diversity among the nutmeg genotypes, classify according to their variations, and examine the genetic relationships among the attributes.

MATERIALS AND METHODS

Present study was carried out in the Horticulture Research Station, Pechiparai, Kanyakumari district between 2019 and 2022. A panel of 25 genotypes were collected from Nagercoil in Kaniyakumari district, Pollachi in Coimbatore district of Tamil Nadu and Kottayam district in Kerala during 2010 and planted in randomized block design with three replications adopting a spacing of 8 m × 8 m. Five nutmeg trees from each replication of each genotype were chosen and tagged in order to record observations on growth and yield features namely plant height (m), stem girth (cm), leaf length (cm), leaf breadth (cm), number of fruits / tree, single fruit weight (g), mace yield per tree (g). Analysis of variance was carried out in order to partition the total variation into variation due to genotypes, replication, year and genotype x year as per Panse and Sukhatame (1967). Genetic variability parameters such as phenotypic and genotypic coefficient of variation (Burton, 1952), heritability and genetic advance as percent of mean (Johnson *et al.,* 1955) were estimated using TNAU STAT (Manivannan, 2014). Path analysis (Dewey and Lu, 1959) was carried out using GRAPES platform version 1.0.0. (Gopinath *et al*., 2020). D^2 analysis were analyzed as per the method suggested by Mahalanobis (1936).

RESULTS AND DISCUSSION

Estimates of variance components: In the present study, 25 genotypes were subjected to pooled ANOVA for seven morphological traits (**Table 1**). It revealed that genotypes and year were highly significant ($p < 0.01$) for all the traits studied indicating the diversity among the genotypes and years studied. Genotype and year interactions were highly significant ($p < 0.01$) for stem girth, number of fruits, single fruit weight and mace yield/tree while significant ($p < 0.05$) for plant height, leaf length and leaf breadth. Significant interactions between genotype and year suggested that genotypes behave differently in various environments.

Highest Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of variation (GCV) was recorded for stem girth (33.69 and 33.48%) and number of fruits (23.73 and 23.28%) (**Table 2**). Mace yield/tree had high PCV (20.30%) coupled with moderate GCV (19.85%). Estimates of PCV and GCV for plant height, leaf length, leaf breadth and single fruit weight were found to be moderate (10-20%). Similarly, moderate to high GCV and PCV has been reported by Vikram *et al.(*2016).

Heritability estimation alone is not as helpful in determining the gain under selection as heritability estimation combined with genetic advance as a percentage of mean (Dhandapani *et al*., 2023). Broad sense heritability was found to be high (> 60 %) (Johnson *et al.,* 1955) for all the studied traits and ranged from 83.50% (leaf length) to 98.73% (stem girth) which depicted that degree of heritability is less affected by the environment (Saha

Table 1. Pooled analysis of variance for the morphological traits studied

Traits	Replication	Genotypes	Year	Y× G	Error
Plant height (m)	0.05	8.24**	99.37**	0.15*	0.04
Stem girth (cm)	2.50**	644.66**	1965.37**	11.73**	1.06
Leaf length (cm)	0.14	15.48**	397.32**	0.59*	0.14
Leaf breadth (cm)	0.05	4.89**	83.82**	0.07*	0.04
No. of fruits	287.08*	52550.28**	355042.84**	814.57**	135.10
Single fruit weight (g)	0.49	143.72**	3964.48**	5.42**	1.74
Mace yield/ tree/ g	77.00*	7829.03**	70455.67**	171.28**	31.53

(*= Significant at p<0.05, **=Significant at p<0.01)

Table 2. Estimation Variability parameters

Characters	GCV	PCV	Heritability in broad sense h^2(%)	Genetic advance as percentage of mean (%)
Plant height	16.14	16.77	92.57	31.99
Stem girth (cm)	33.48	33.69	98.73	68.53
Leaf length (cm)	11.29	12.35	83.50	21.25
Leaf breadth (cm)	14.21	14.87	91.39	27.99
No. of fruits	23.28	23.73	96.27	47.06
Single fruit weight (g)	10.58	11.49	84.83	20.09
Mace yield/ tree/ g	19.85	20.30	95.68	40.01

et al., 2019). Genetic advance as percent of mean ranged from 20.09% (single fruit weight) to 68.53% (stem girth). High genetic advance as percent of mean was identified for all the studied variables, concomitant with high heritability values. Greater heritability, genetic advance and superior GCV provide a more accurate sign of selection than particular genetic matrices (Khan *et al.,* 2020). Thus, the selection programme for nutmeg might be based on the number of fruits, stem girth and mace yield/tree. There has been evidence of high variability for fruit, mace, and nut traits in nutmeg genotypes in earlier studies by Haldankar *et al.* (2004), Kumar *et al.*(2010) and Vikram *et al*.(2016).

Path analysis: Path analysis divides predictor variable correlation coefficients into direct and indirect effects on the dependent variable, in this instance mace yield. Present study revealed that number of fruits had high positive direct effect on mace yield while single fruit weight and plant height were found to have moderate direct effect on mace yield (**Table 3, Fig. 1**). The trait leaf length showed positive indirect effect while stem girth negative indirect effect on mace yield through number of fruits. All other traits showed negligible effect on mace yield through indirect effects. It could be inferred that number of fruits, single fruit weight, stem girth and plant height contributed equally through direct and indirect effects for

Table 3. Direct and indirect effect of various characters for nutmeg genotypes

Variables	Plant height (m)	Stem girth (cm)	Leaf length (cm)	Leaf breadth (cm)	No. of fruits	Single fruit weight (g)
Plant height (m)	**0.221**	-0.008	-0.001	0.01	-0.142	-0.039
Stem girth (cm)	0.128	**-0.014**	0.01	0.007	-0.223	-0.015
Leaf length (cm)	0.003	0.002	**-0.058**	0.013	0.102	0.08
Leaf breadth (cm)	0.021	-0.001	-0.007	**0.103**	0.012	0.099
No. of fruits	-0.077	0.008	-0.014	0.003	**0.411**	0.013
Single fruit weight (g)	-0.032	0.001	-0.018	0.038	0.02	**0.265**

Residual effect : 0.7603

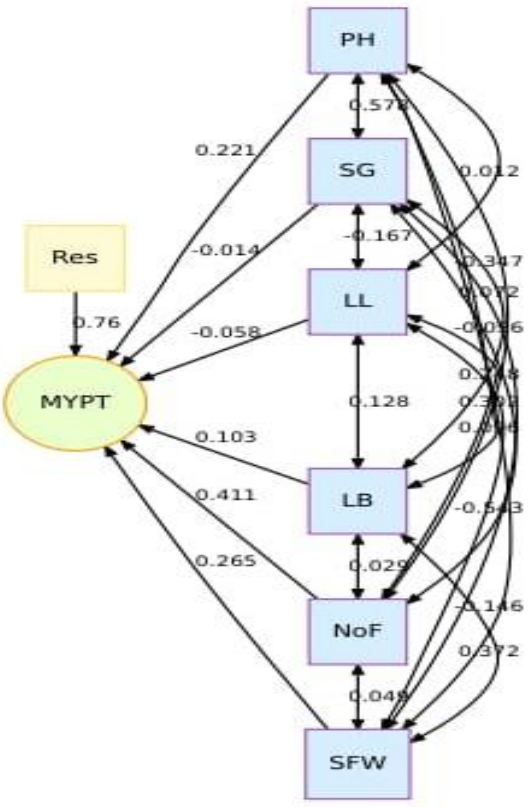

Fig. 1. Path analysis diagram

mace yield improvement. Direct selection of these traits would result in identification of higher mace yield nutmeg genotypes (Manivannan *et al.,* 2018; Shanmugam *et al.,* 2024; Tolera *et al.,* 2024).

Cluster analysis: Genetic diversity was assessed using data obtained from seven traits in 25 nutmeg genotypes using Mahalanobis D^2 analysis. It indicated that genotypes were grouped in to four clusters. The clustering pattern revealed that Cluster I had 16 genotypes, forming the largest cluster (**Table 4**). Cluster III had six genotypes followed by cluster IV which had two genotypes (MF-15 and MF-19). Cluster II was found to be a solitary cluster (MF-4), suggesting that it might possess unique characteristics compared to the other clusters. The intra-cluster D^2 values ranged from 0 to 253.29 (**Table 5**). The maximum intra-cluster distance was found within cluster I (253.29) followed by cluster III (203.87). Clusters II had zero intra-cluster distance, as they had only one nutmeg genotype. High intra-cluster distance found in cluster I indicated a high degree of genetic variation among the nutmeg genotypes in that cluster. Maximum inter-cluster distance was observed between clusters I and II (1640.91) followed by clusters II and III (800.59). This indicates that there was a considerable degree of genetic diversity among the genotypes in these clusters. Minimum inter-cluster distance was observed between the cluster III and IV (340.58) which indicating the close association and similarity between the nutmeg genotypes for most of the characters in these clusters. These

Table 4. Composition of D^2 clusters for nutmeg genotypes based on their traits

Clusters	Genotypes
Cluster I	MF-1, MF-2, MF-5, MF-6, MF-10, MF-12, MF-11, MF-7, MF-8, MF-3, MF-9, MF-13, MF-20, MF-25, MF-17, MF-18
Cluster II	MF-4
Cluster III	MF-14, MF-23, MF-21, MF-24, MF-16, MF-22
Cluster IV	MF-15, MF-19

Table 5. Inter and intra cluster distance for nutmeg genotypes

Cluster	I	II	III	IV
I	253.29	1640.91	612.06	401.34
II		0.00	800.59	742.20
III			203.87	340.58
IV				188.23

* Diagonal values indicate intra cluster distance

results were in accordance with Behera *et al.* (2018) and Lakshmi *et al.* (2022).

The cluster mean value for plant height ranged from 6.90m (cluster II) to 8.30 (cluster I) (**Table 6**). For stem girth, it ranged from 19.27cm (cluster II) to 41.23cm (cluster I), for leaf length it ranged from 15.30cm (cluster III) to 19.68cm (cluster II), for leaf breadth it ranged from 6.41cm (cluster IV) to 9.95cm (cluster II), for number of fruits it ranged from 55.93 (cluster IV) to 708.51 (cluster II), for single fruit weight it ranged from 46.03g (cluster IV) to 60.77g (cluster II) and for mace yield per tree it ranged from 194.61g (cluster I) to 299.73g (cluster II). Apart from divergence, it is also important to gain an understanding of the relative contribution of these for targeted breeding programs aimed at enhancing specific characteristics in nutmeg varieties ultimately optimizing overall agronomic performance and yield (Patil *et al.,* 2013). Of the traits studied, stem girth exhibited the maximum contribution (37.66%) towards the diversity followed by mace yield/ tree (33.33%). The least contribution was by leaf length (**Table 7**). This underscores the significance of stem girth and mace yield per tree in delineating genetic divergence among nutmeg genotypes

From the present study, it was concluded that number of fruits, stem girth and mace yield/tree showed high GCV, PCV, heritability coupled with high genetic advance as

Table 6. Cluster mean for nutmeg traits

Cluster	Plant height	Stem girth	Leaf length	Leaf breadth	Number of fruits	Single fruit weight	Mace yield/ tree
I	8.30	41.23	15.17	7.24	435.94	48.30	194.61
II	6.90	24.37	19.68	9.95	708.51	60.77	299.73
III	6.96	19.27	15.30	7.18	490.19	49.42	195.33
IV	7.60	34.19	15.85	6.41	55.93	46.03	250.23

Table 7. Contribution of traits towards genetic divergence in nutmeg genotypes

Character	Rank	Contribution in percentage (%)
Plant height (m)	18	6
Stem girth (cm)	113	37.66
Leaf length (cm)	7	2.33
Leaf breadth (cm)	10	3.33
No. of fruits	45	15
Single fruit weight (g)	7	2.33
Mace yield/ tree/ g	100	33.33

percent of mean. Therefore, in choosing the genotypes to increase the yield, these features should be taken into account. Path analysis revealed that the traits, number of fruits, single fruit weight, stem girth and plant height contributed equally through direct and indirect effects for mace yield improvement. Based on the diversity studies, the traits stem girth and mace yield/tree were identified as important traits that contributed maximum for the divergence among the genotypes. These findings contribute to a comprehensive understanding of the relationships among nutmeg genotypes, aiding in the identification of divergent and potentially complementary traits for targeted breeding and improvement efforts.

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