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Genetic diversity analysis of indigenous turmeric genotypes using horticultural markers

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ABSTRACT

The genetic diversity among 25 turmeric genotypes was evaluated for 18 horticultural traits. Ward's minimum variance cluster analysis based on squared Euclidian's distance on different traits clearly separated six clusters. The maximum intra-cluster distance was observed in cluster III (68.89) followed by cluster I (51.15), on the other hand, the highest Euclidean's inter-cluster distance was observed between clusters I and VI (332.26) followed by clusters II and III (205.26). The highest percent contribution towards genetic divergence was noticed with days to maturity followed by curcumin content. Based on principal component analysis (PCA), the first five components explained 89.83% of total genetic variation. As a result, genetically diverse genotypes could be identified, increasing the usefulness of genotype collections by broadening the genetic base of turmeric and to utilize them in crop improvement programmes through direct selection.

Key words: *Curcuma longa* L., D^2 analysis, morphological traits, principal component analysis.

INTRODUCTION

Turmeric, a perennial rhizomatous herbaceous plant of the genus *Curcuma*, is cultivated extensively in the tropics for its rhizomes having culinary and medicinal importance. Turmeric is also commonly known as 'Golden spice', and considered to be a triploid species (2n = 3x = 63; x = 21) (Ramachandran, 11). India is the largest producer, consumer and exporter of turmeric in the world, with an annual production of about 1,190 thousand tonnes from an area of 233 thousand ha and productivity of 5.11 tonnes/ ha (2013-2014). Tamil Nadu is the foremost state in turmeric production with an area of 77 thousand ha and a production of 462 thousand tonnes, followed by Telangana, Andhra Pradesh and Karnataka (Anon, 2). Though turmeric is propagated clonally using its underground rhizomes, viable sexual reproduction is also reported (Siju *et al*., 14). Curcumin, the mayer bioactive components of turmeric, is a powerful antioxidant, anti-parasitic, antispasmodic and anti-inflammatory compounds that can also inhibit carcinogenesis and cancer growth (Araujo and Leon, 3).

Since, it is gaining high demand by the food, cosmetic and pharmaceutical industries, turmeric crops have been the focus of different studies (May *et al*., 8). However, to obtain further increase in productivity, information regarding the crop's genetic diversity is needed for breeding programmes (Sigrist *et al*., 13). Various multivariate methods can be applied to compile knowledge on genetic diversity. The most commonly used methods by breeders are analysis by principal component analysis (PCA), analysis of canonical variables and the clustering methods (Mohammadi and Prasanna, 9). PCA is a multivariate technique that analyzes a series of data, which observations are described by several inter-correlated quantitative dependent variables (Abdi and Williams, 1). Mahalanobis D^2 statistics is a powerful tool for measuring divergence among a set of population on the basis of statistical distance utilizing multivariate measurements. Therefore, the present study was undertaken to estimate the diversity among turmeric genotypes in order to develop selection criteria for improving rhizome yield in turmeric.

MATERIALS AND METHODS

Twenty-five indigenous turmeric genotypes were grown during 2008-09 and 2009-10 at Vegetable Research Farm, Department of Horticulture, SHIATS, Allahabad. The genotypes were collected from Narendra Deva University of Agriculture and Technology, Faizabad, Uttar Pradesh. The experiment was laid out in randomized complete block design (RCBD) with three replications. The rhizomes were planted on ridges adopting a spacing of 45 cm × 25 cm between rows and plants, respectively ensuring 12 plants in each plot. All recommended cultivation package and protective measures were followed to raise a healthy crop. The data were recorded from five randomly selected plants from each treatment

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in each replication and mean data was subjected for statistical analysis for 18 diverse traits, *viz*., days to sprouting, plant height (cm), number of leaves per plant, number of tillers per plant, days to maturity, weight of rhizomes per plant (g), weight of primary rhizomes per plant (g), weight of secondary rhizomes per plant (g), number of primary rhizomes per plant, number of secondary rhizomes per plant, diameter of mother rhizome (cm), diameter of primary rhizome (cm), diameter of secondary rhizome (cm), length of primary rhizome (cm), length of secondary rhizome (cm), dry matter recovery (q/ ha), curcumin content (%) and rhizome yield (q/ ha).

Curcumin content was estimated by taking absorbance as the intensity of yellow colour at 425 nm on a spectrophotometer. For reference, 0.42 absorbance at 425 nm was taken as 0.0025 g curcumin.

$$\text{Curcumin content (g/100 g)} = \frac{0.0025 \times A425 \times \text{volume made up} \times \text{dilution factor} \times 100}{0.42 \times \text{weight of the sample (g)} \times 1000}$$

The mean value of these plants was computed and used for statistical analysis. Analysis of variance was carried out as per standard method (Panse and Sukhatme, 10). Mean data for each traits was subjected to multivariate analysis utilizing Mahalanobis's D^2 statistic (Mahalanobis, 7) and Rao (12) using statistical software WINDOSTAT 9.2.

RESULTS AND DISCUSSION

Pooled analysis of variance revealed highly significant mean square values of genotypes for all the traits studied indicates the existence of sufficient genetic variability among the studied genotypes (Table 1). The dendrogram obtained from the cluster analysis formed by Euclidean's method grouped the 25 turmeric genotypes into two main-clusters (Fig. 1). The first major cluster had 23 genotypes, while the second cluster consisted of two genotypes. The first major cluster was divided further into two sub-clusters. The first sub-cluster included four genotypes, whereas, the second sub-cluster had 19 genotypes. The second sub-cluster of first major cluster was further divided into two sub-sub-clusters. The first sub-sub-cluster consisted of seven genotypes, while second sub-sub-cluster had twelve genotypes. The first sub-sub-cluster was further divided into two sub-sub-subgroups. The first sub-sub-subgroups had three, while second had four genotypes, similarly the second sub-sub-cluster also divided into two sub-sub-subgroups, the first sub-sub-subgroups has eight, whereas, second had four genotypes. Finally, 25

Table 1. Pooled analysis of variance for eighteen traits of turmeric genotypes.

Trait	Mean sum of squares		
	Replication (d.f.= 2)	Treatment (d.f.= 24)	Error (d.f. = 120)
Days to sprouting	0.88	17.82**	0.65
Plant height (cm)	4.60	31.99**	1.22
No. of leaves per plant	0.46	5.98**	1.04
No. of tillers per plant	1.96	0.67**	0.15
Days to maturity	0.35	173.32**	2.63
Weight of rhizomes per plant (g)	1694.37	2659.37**	621.29
Weight of primary rhizomes per plant (g)	1980.48	1555.07**	222.98
Weight of secondary rhizomes per plant (g)	85.17	140.17**	16.24
No. of primary rhizomes per plant	6.34	0.58**	0.39
No. of secondary rhizomes per plant	3.24	6.31**	0.79
Diameter of mother rhizome (cm)	1.35	1.14**	0.07
Diameter of primary rhizome (cm)	0.04	0.42**	0.10
Diameter of secondary rhizome (cm)	0.55	1.59**	0.24
Length of primary rhizome (cm)	1.65	5.87**	0.59
Length of secondary rhizome (cm)	3.12	8.40**	0.33
Dry matter recovery (q/ ha)	0.18	1.42**	0.06
Curcumin content (%)	1.28	5.79**	0.34
Rhizomes yield (q/ ha)	1.40	14.33**	0.31

**Significant at 0.1%

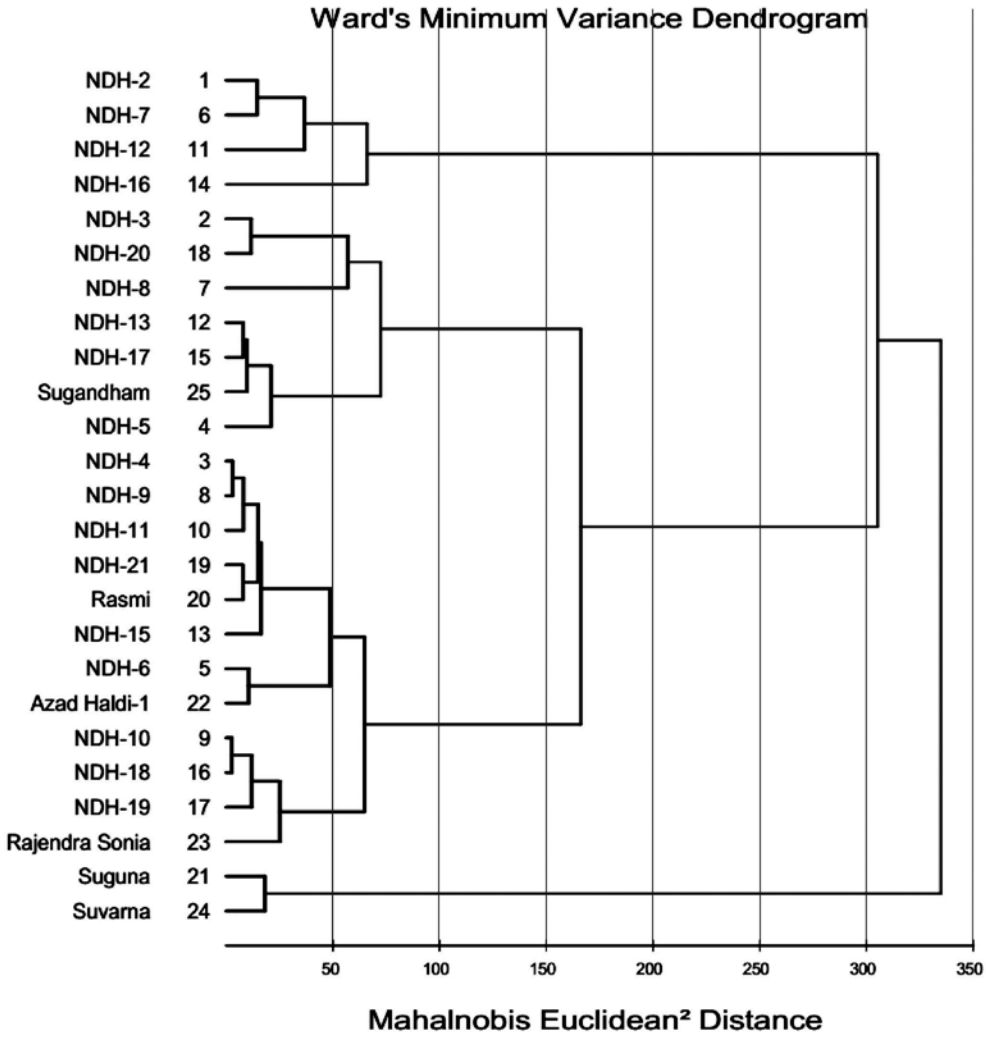

Fig. 1. Dendrogram depicting classification of 25 turmeric genotypes based on 18 horticultural traits.

genotypes were divided into six clusters. Cluster IV (32%) comprising eight genotypes was the biggest and showed high homogeneity among them or the least genetic variation, followed by clusters I, III, and V (16% each) each containing four genotypes, cluster II (12%) comprises three genotypes, cluster VI (8%) was smallest with two genotypes only (Table 2). Pattern of distribution of genotypes among various clusters reflected the significant genetic variability present in the genotypes tested. The clustering of the genotypes indicated no parallelism between genetic diversity and geographical diversity. In line with this, Cintra *et at*. (5) grouped 21 turmeric genotypes into five clusters. Similarly, Verma *et al*. (15) clustered 83 turmeric genotypes into 10 clusters using Mahalanobis's distance.

Euclidean's average intra- and inter-cluster D^2 values are tabulated in Table 3, providing information on the nature of genetic divergence present at intra- and inter-cluster levels, respectively. In general, inter-cluster distances were much higher than those of intra-cluster distances, suggesting homogeneous and heterogeneous nature of the genotypes within and between the clusters, respectively. The intra-cluster distance was maximum in cluster III (68.89), revealing considerable genetic divergence within the genotypes of this cluster and was due to both natural and artificial selection forces among the genotypes, followed by cluster I (51.15), cluster V (38.85), whereas minimum was found in cluster II (0) followed by cluster IV (25.75) and cluster VI (36.54). The intra-cluster distance indicated that the genotypes within the clusters were less diverse. The highest inter-cluster distance was observed between cluster I and VI (332.26) followed by cluster II and III (205.26), cluster IV and VI (228.09), cluster II

Table 2. Distributions of 25 turmeric genotypes in to different cluster groups.

Cluster No.	No. of genotypes	Genotype(s)
I	4	NDH-2, NDH-7, NDH-12, NDH-16
II	3	NDH-3, NDH-20, NDH-8
III	4	NDH-13, NDH-17, Sugandham, NDH-5
IV	8	NDH-4, NDH-9, NDH-11, NDH-21, Rasmi, NDH-15, NDH-6, Azad Haldi-1
V	4	NDH-10, NDH-18, NDH-19, Rajendra Sonia
VI	2	Suguna, Suvarna

Table 3. Euclidean intra- (diagonal) and inter- (off diagonal) cluster distance of 25 turmeric genotypes based on 18 horticultural traits (over the years).

Cluster	I	II	III	IV	V	VI
I	51.15	105.09	161.18	71.17	161.48	332.26
II		0.00	205.26	115.93	190.34	123.34
III			68.69	74.73	104.27	257.93
IV				25.75	63.63	228.09
V					38.85	214.27
VI						36.54

and V (190.34), and lowest inter-cluster distance was observed between cluster III and IV (74.73) followed by cluster I and IV (71.17), cluster III and V (104.27), cluster IV and V (63.63) . The highest inter-cluster distance between cluster I and VI indicted that the genotypes belonging to these clusters were genetically diverse and used as potential parents for future breeding programmes. Similarly, the lowest inter-cluster distance between cluster III and IV indicted the genotypes belonging to each pair of cluster were less diverse.

Among the 18 characters, days to maturity contributed maximum (47%) towards genetic divergence followed by curcumin content (12.33%), diameter of mother rhizome (10%), plant height (9.33%) and days to sprouting (6.33%), indicating that these characters contributing most of the divergence and importance should be given for effective selection. Diameter of primary rhizome and length of secondary rhizome contributed 3.67% of divergence each. Rhizome yield and weight of primary rhizomes per plant contributed 3.0 and 1.33%, respectively, towards genetic divergence. Number of secondary rhizomes per plant and diameter of secondary rhizome contributed 1.0% each towards genetic divergence. On the other hand, traits like weight of secondary rhizomes per plant and length of primary rhizome contributed 0.67% of divergence each. Remaining traits, *viz*., number of leaves, number of tillers, weight of rhizomes per plant, number of primary rhizomes per plant and dry matter recovery did not show any contribution (0) towards genetic divergence. Cintra *et al*. (5) reported that curcuminoid content was the trait that contributed most to the genetic divergence (64.77). Verma *et al*. (15) reported that the highest percentage contribution of genetic divergence in turmeric was by rhizome yield (64.68) followed by weight of primary rhizome (19.37) and plant height (10.84).

Based on average values of each cluster for the measured traits, the characteristics of early maturity (154.71 days) and highest cucurmin content (4.91%) were observed in cluster I than the other clusters. Hence, genotypes from this cluster can be very useful developing early maturing and high cucurmin content genotypes. Maximum weight of primary rhizomes per plant (78.41 g), weight of secondary rhizomes per plant (13.78 g), number of primary rhizomes per plant (3.54), number of secondary rhizomes per plant (2.72), diameter of mother rhizome (3.48 cm), diameter of primary rhizome (1.87 cm), diameter of secondary rhizome (1.17 cm), and length of secondary rhizome (3.63 cm) were noticed in cluster III, whereas, having minimum number of leaves per plant (9.24), number of tillers (1.79) and weight of rhizomes per plant (161.63 g). In the line with the present finding, Chaveerach *et al*. (4) reported that in *Curcuma sattayasaii*, secondary rhizome cylinderis 1.0-1.5 cm in diameter. Cluster

V having the characteristics of taking maximum days to sprouting and maturity (24.80; 168 days, respectively) as compared to other clusters. For crop improvement in turmeric, plant height and number of leaves determines the yield potential of the genotypes. Cluster VI, which comprised the highest yielding genotypes, characteristics of early sprouting (19.5 days), maximum plant height (25.5 cm), number of leaves (12), number of tillers (2.81), weight of rhizomes per plant (215.85 g), length of primary rhizome (6.8 cm), dry matter recovery (46.73 q/ ha) and yield per ha (173.68 q), considering yield, this cluster can be used to develop high yielding as well as higher weight of rhizomes per plant, dry matter recovery, plant height and early sprouting genotypes. Similar results were found in earlier attempts of Chaveerach *et al*. (4) and Jan *et al*. (6), who reported that main rhizome ranged from 5-10 cm long.

Principal component analysis reveals the significance of the major contributor to the total variation at each axis of differentiation. The eigen values helps in determining the number of factors to be retained. The results of the PCA of the genotypes of turmeric are presented in Table 4. The principal component analysis revealed that five principal components PC1, PC2, PC3, PC4 and PC5 with eigen values 6.706, 4.295, 3.181, 1.212 and 0.777, respectively have accounted for 89.83% of the total variation. The first principal component (PC1) accounted for 37.25% of the total variation for which days to sprouting, plant height, number of leaves, number of tillers, weight of rhizomes per plant, length of primary rhizome, dry matter recovery and rhizome yield had the highest loading. Principal component 2 accounted for 23.86% of variation; weight of secondary rhizomes per plant, diameter of secondary rhizome and length of secondary rhizome had the maximum eigen vectors value in PC2. In the third PC, days to maturity, weight of primary rhizomes per plant, number of primary rhizomes per plant and diameter of mother rhizome had the maximum eigen vectors. This PC accounted for 17.67% of the total variation. Principal component 4 accounted for 6.73% of variation and number of tillers, number of leaves per plant and dry matter recovery had the maximum positive eigen vectors in this PC, but values

Table 4. Principal component analysis for different traits in turmeric genotypes (over the years).

Variable	PC of the accessions				
	PC1	PC2	PC3	PC4	PC5
Days to sprouting	0.338	0.065	0.127	0.173	0.226
Plant height (cm)	-0.369	-0.021	-0.035	-0.101	0.067
No. of leaves per plant	-0.326	-0.001	0.056	0.242	-0.015
No. of tillers per plant	-0.317	0.046	0.024	0.284	0.028
Days to maturity	-0.047	0.315	0.354	-0.236	-0.099
Weight of rhizomes per plant (g)	-0.374	-0.020	0.006	-0.121	0.064
Weight of primary rhizomes per plant (g)	-0.141	0.082	-0.404	-0.361	0.010
Weight of secondary rhizomes per plant (g)	0.046	0.401	-0.261	-0.055	-0.039
No. of primary rhizomes per plant	-0.004	-0.178	-0.481	-0.059	-0.175
No. of secondary rhizomes per plant	-0.034	0.393	-0.018	0.216	0.473
Diameter of mother rhizome (cm)	-0.007	0.285	-0.374	-0.234	-0.063
Diameter of primary rhizome (cm)	-0.018	0.265	0.326	-0.291	-0.378
Diameter of secondary rhizome (cm)	0.091	0.420	0.151	-0.030	-0.196
Length of primary rhizome (cm)	-0.308	0.256	0.071	0.082	-0.002
Length of secondary rhizome (cm)	0.105	0.355	-0.271	0.135	0.285
Dry matter recovery (q/ ha)	-0.358	0.021	0.046	0.236	0.085
Curcumin content (%)	-0.072	-0.143	0.201	-0.583	0.620
Rhizome yield (q/ ha)	-0.360	-0.014	-0.031	-0.071	-0.125
Eigen value	6.706	4.295	3.181	1.212	0.777
Partial variance (%)	37.256	23.860	17.673	6.733	4.316
Cumulative variance (%)	37.256	61.116	78.789	85.522	89.838

were less than other PCs. The major contributing character for the diversity in the principal component 5 (PC5) were number of secondary rhizome per plant, diameter of primary rhizome and curcumin content. This PC accounted for 4.31% of the total variation. Conventionally, one variable is selected from these identified groups depending on respective loadings. Therefore, for the first group rhizome yield was the best choice, which had the largest loading from PC1, weight of secondary rhizomes per plant for PC2, days to maturity for PC3, and curcumin content for fifth group (PC5). The present study confirms that cluster analysis has proved to be effective method in grouping turmeric genotypes that may facilitate their effective utilization in crop improvement programmes through clonal selection, as conventional breeding through hybridization is difficult in this crop.

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