



Genetic Divergence among *Curcuma longa* L. accessions

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ABSTRACT - *The genetic divergence among 21 turmeric genotypes was evaluated based on morpho-agronomical traits and on multivariate procedures in order to select divergent top-yielding genotypes. The experiment was carried out from 2001/2003 in randomized block design with four replications. Six descriptors describing rhizome production and curcuminoid content were evaluated. From Mahalanobis' distance matrix were applied Tocher's clustering algorithm and single linkage clustering and analysis of canonical variables. The relative importance of the descriptors was also evaluated. Multivariate analysis techniques allowed an effective study of genetic divergence and the grouping of the 21 accessions into five clusters. Curcuminoid content and dry weight were the traits that contributed most to genetic divergence and allowed selection of the best accessions for breeding programs to develop high-yield genotypes with high contents of curcuminoids.*

INTRODUCTION

Turmeric (*Curcuma longa* L.) is a plant of the Zingiberaceae family and comprises about 70 species (Smartt and Simmonds 1992). It is an herbaceous and perennial rhizome plant, native of India, where it is concentrated in greatest variability. The plant is vegetatively propagated by the rhizomes. These grow in an organized structure, below the stem of the plant, where the smaller so-called "finger" rhizomes are grouped about a larger one called top or head. The commercial interest focuses on these rhizomes of the crop (Cecílio Filho and Souza 1999).

Presently, three turmeric products are commercially available: turmeric powder, turmeric oleoresin and curcumin

extract (Govindarajan 1980). The crop plays an important role in food industry, where it has been conquering the world market as a solution for the substitution of synthetic coloring, besides being used for its medicinal and pharmacological qualities (Scartezzini and Speroni 2000).

With this economical importance and diversity of use turmeric production has a great growth potential. In Brazil, turmeric is most intensely grown in the region of Mara Rosa, state of Goiás. The improvement of cultivation practices however still lacks basic information on the crop and the management of genetic variability of genotypes found in the country. On this background, more attention should be paid to the crop in the sense of optimizing the exploitation of genetically available resources (Pinheiro et al. 2003).

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Few studies found in literature deal with genetic variability in turmeric. Radhakrishnan et al. (1995) evaluated six turmeric cultivars, showing that cultivars with high indices of green matter yield presented low curcumin contents after drying, indicating a negative correlation between these traits. Yadav et al. (1996) evaluated 17 turmeric genotypes grown in the dry season and observed significant differences for all traits evaluated with exception for leaf width. Pinheiro et al. (2003) analyzed the genetic divergence in 20 accessions based on molecular RAPD markers, and showed a small divergence between accessions collected in Mara Rosa, GO, suggesting that the producers of the township used a mixture of genotypes for commercial planting.

Various multivariate methods can be applied to compile knowledge on genetic diversity. The most commonly used methods by breeders are analysis by main components, analysis by canonic variables and the agglomerative methods, among others. The choice of the most adequate method has been determined depending on the precision desired by the researcher, the easiness of analysis and the way the data were obtained (Cruz and Regazzi 1997).

In this context, our study had the main objective of estimating the genetic divergence of 21 turmeric genotypes by the evaluation of seven morpho-agronomical descriptors aiming at the identification of the most promising genotypes for the crop and to use them in programs of genetic improvement. Furthermore, the relative contribution of these descriptors to the genetic divergence was evaluated.

MATERIAL AND METHODS

The underlying data were obtained in an experiment realized from October 2001 to July 2003. The yield data were evaluated as a two-year field study. Twenty-one accessions from the states of Goiás, São Paulo and Minas Gerais (Table 1) were used in an experimental design of complete randomized blocks with 21 treatments and four replications. The experimental plot was represented by three rows of three meters. Accession eight (Santa Rosa) was replicated only twice. Rows were spaced 0.45 m, and plants 0.20 m apart. Data were obtained from the two central meters of the central row (useful plot) considering the mean of the plants in the useful plot. The following descriptors were evaluated: number of heads (NH), weight

heads (WH), number of fingers (NF), finger weight (FW), total weight (TW), dry weight (DW) and curcuminoid content (CC). Extraction and determination of the curcuminoid content were realized according to the norm NBR 13624 (ABNT 1996). The data were subjected to analysis of variance to verify the existence of genetic variability among the accessions and the means were compared by the test of Scott-Knott at 5% probability (Scott and Knott 1974).

A multivariate analysis was performed, applying the techniques of clustering and of canonical variables. In the clustering technique by the criterion of the nearest neighbor, Mahalanobis' generalized distance was used as measure of dissimilarity and the method of optimization of Tocher, cited by Rao (1952), for the delimitation of groups. In the analysis of canonical variables, the genetic divergence was evidenced by display of dispersion, where the axes were represented by the first canonical variables. The relative importance of each canonical variable and of the descriptors (Cruz and Regazzi 1997) in the prediction of genetic divergence were also studied. The statistical analyses were processed on software Genes (Cruz 1997).

RESULTS AND DISCUSSION

The summary of analysis of variance for the seven descriptors under evaluation is presented in Table 2. Results indicate statistically significant differences ($P < 0.01$) among the accessions for DW and CC and ND at a level of 5% significance. For the descriptors NH, WH, FW, and TW, the analysis of variance did not evidence significant difference among the accession means. Results show that the turmeric accessions generally presented a small genetic variability limiting the possibilities with crop improvement. The experimental variation coefficient values can be considered satisfactory for the evaluated descriptors, with a lower value for trait CC.

The means of the 21 turmeric accessions, considering the seven evaluated descriptors, were compared using the Test of Scott-Knott at 5% probability (Table 3). In concordance with the analysis of variance the descriptors NH, WH, ND, FW, and TW did not present significant differences. Two groups were formed for DW; the group with the higher means was consisted of accessions 19, 20 and 21. Three groups were formed for CC: 10 accessions formed the group with the highest means, varying from 10.14 to 11.27%; nine accessions formed an intermediate group with means varying from 9.21

Table 1. Identification of the accessions with their respective origin

Accession	Origin
1	Alexânia – GO
2	Goiânia – GO
3	Botucatu – SP
4	Ibitinga – SP
5	Lavras – MG
6	Campinas – SP (IAC ⁺) ¹
7	Goiânia – GO
8	Mara Rosa – GO
9	Mara Rosa – GO
10	Rubiataba – GO
11	Mara Rosa – GO
12	Mara Rosa – GO
13	Mara Rosa – GO
14	Mara Rosa – GO
15	Mara Rosa – GO
16	Mara Rosa – GO
17	Mara Rosa – GO
18	Mara Rosa – GO
19	Mara Rosa – GO
20	Campinas – SP (IAC ⁻) ²
21	Mara Rosa – GO

¹ Selection performed at the Instituto Agronômico de Campinas for higher curcumin content

² Selection performed at the Instituto Agronômico e Campinas for lower curcumin content

Table 2. Summary of the analysis of variance for seven descriptors evaluated in 21 turmeric accessions over two years

Sources of variation	df	NC ¹	PC	ND	FW	TW	DW	CC
Blocks	2	554.3333	0.0109	2410.7778	0.4381	0.5775	0.0086	0.1939
Accessions	20	416.7333	0.1075	26165.444*	1.4347	2.1966	0.1790**	23.8819**
Error	40	232.7667	2.8539	14109.7611	0.8508	1.2943	0.0674	0.2219
Means		60.3333	1.1597	555.7778	3.2919	4.4516	1.0959	9.1709
CV (%)		25.2873	23.0320	21.3727	28.0202	25.5566	23.6805	5.1396

¹ NH: number of heads, WH: weight heads in kg plot⁻¹, ND: number of fingers in kg plot⁻¹, FW: finger weight in kg plot⁻¹, TW: total weight in kg plot⁻¹, DW: dry weight in kg plot⁻¹ and CC: curcuminoid content in %

*, ** P < 0.05 and P < 0.01, respectively, by the F test

Table 3. Means of the 21 turmeric accessions related to seven descriptors evaluated over two years

Accessions	NC ¹	PC	ND	FW	TW	DW	CC
1	58.33 a	1.08 a	425.00 a	2.23 a	3.32 a	0.77 b	10.57 a
2	52.67 a	0.84 a	461.00 a	2.64 a	3.48 a	0.82 b	9.92 b
3	60.33 a	0.97 a	463.00 a	2.72 a	3.69 a	0.89 b	10.21 a
4	67.00 a	1.03 a	559.00 a	2.64 a	3.67 a	0.94 b	9.76 b
5	51.00 a	0.78 a	384.33 a	2.18 a	2.96 a	0.74 b	9.57 b
6	58.33 a	1.08 a	603.33 a	3.64 a	4.73 a	1.06 b	9.30 b
7	60.00 a	1.15 a	504.00 a	2.87 a	4.02 a	0.98 b	10.43 a
8	64.33 a	1.26 a	515.00 a	3.26 a	4.51 a	1.08 b	11.27 a
9	72.67 a	1.33 a	673.33 a	3.75 a	5.08 a	1.19 b	9.70 b
10	76.67 a	1.20 a	538.00 a	3.06 a	4.27 a	1.02 b	9.28 b
11	64.33 a	1.26 a	593.67 a	3.71 a	4.97 a	1.15 b	9.94 b
12	55.67 a	1.17 a	506.00 a	3.06 a	4.23 a	1.08 b	10.60 a
13	66.00 a	1.11 a	491.67 a	2.78 a	3.89 a	0.94 b	9.21 b
14	56.67 a	1.04 a	522.67 a	3.08 a	4.12 a	1.01 b	10.55 a
15	70.67 a	1.31 a	549.00 a	3.54 a	4.84 a	1.20 b	10.14 a
16	70.00 a	1.36 a	618.00 a	3.97 a	5.33 a	1.26 b	10.25 a
17	57.33 a	1.06 a	549.00 a	3.35 a	4.40 a	1.05 b	10.46 a
18	65.00 a	1.17 a	589.33 a	3.45 a	4.62 a	1.16 b	9.70 b
19	75.33 a	1.63 a	762.00 a	4.71 a	6.35 a	1.55 a	10.31 a
20	36.67 a	1.36 a	704.33 a	4.76 a	6.12 a	1.75 a	0.96 c
21	28.00 a	1.18 a	659.67 a	3.74 a	4.92 a	1.38 a	0.69 c

¹ NH: number of heads, WH: weight heads in kg plot⁻¹, ND: number of fingers in kg plot⁻¹, FW: finger weight in kg plot⁻¹, TW: total weight in kg plot⁻¹, DW: dry weight in kg plot⁻¹ and CC: curcuminoid content in %
Means followed by the same letter did not differ from each other by the test of Scott-Knott at 5% probability

Table 4. Divergent groups among 21 turmeric genotypes provided by Tocher' algorithm applied on Mahalanobis' distance matrix

Group	Genotypes ¹
I	14 17 3 7 16 11 15 2 12
II	10 13 18 9 4 6 5
III	1 8
IV	20 21
V	19

¹ Genotypes: 1 Alexânia – GO, 2 Goiânia – GO, 3 Botucatu – SP, 4 Ibitinga – SP, 5 Lavras – MG, 6 Campinas – SP (IAC²), 7 Goiânia – GO, 8 Mara Rosa – GO, 9 Mara Rosa – GO, 10 Rubiataba – GO, 11 Mara Rosa – GO, 12 Mara Rosa – GO, 13 Mara Rosa – GO, 14 Mara Rosa – GO, 15 Mara Rosa – GO, 16 Mara Rosa – GO, 17 Mara Rosa – GO, 18 Mara Rosa – GO, 19 Mara Rosa – GO, 20 Campinas – SP (IAC²), 21 Mara Rosa – GO.

to 9.94%; and accessions 20 and 21 formed another group with lower means, with values between 0.69 and 0.96%, respectively. Accession 19 was noted for the most favorable performance, mainly in terms of DW and CC, with means of 1550 kg plot⁻¹ and 10.3%, respectively. Both traits are important for turmeric yield evaluation, indicating the great potential for cultivation and use in improvement programs this genotype holds. Accessions 20 and 21 presented DW high means, but have nearly no curcuminoids. This material could be selected and improved as an alternative starch source (Leonel and Cereda 2002).

Accessions 20 and 21 were the ones that presented the greatest distances in relation to the other accessions, while the lowest value was observed between accessions 14 and 17. Table 4 lists the groups resulting from the application of the algorithm of Tocher considering Mahalanobis' distance.

The estimates of the genetic distances allowed the formation of five distinct groups by the method of Tocher. Nine accessions formed group I; seven accessions group II; groups III and IV were formed by two accessions each, and accession 19, once more outstanding, formed group V alone. This grouping indicates that the majority of the accessions collected in Mara Rosa, main representatives of groups I and II, have a low genetic divergence, corroborating the results obtained by Pinheiro et al. (2003) who worked basically with the same group of accessions and verified the low genetic dissimilarity through RAPD markers.

Table 5. Relative contribution of the seven descriptors to the genetic divergence of 21 turmeric genotypes using the Singh (1981)' criterion

Descriptor	Value (%)
Number of heads	3.69
Weight of heads	0.82
Number of fingers	2.65
Weight of fingers	4.33
Total weight	5.03
Dry weight	18.71
Curcuminoid content	64.77

The dendrogram (Figure 1) formed by the nearest neighbor criterion based on the underlying genetic distances between groups did not allow a clear visualization of the accession groups formed. The great divergence of accessions 20 and 21 in relation to the others had great influence on the obtained results, making a clear comparison between the two clustering criteria difficult (criterion of the nearest neighbor and the method of Tocher). Nevertheless, it was still possible to verify in the dendrogram that, besides the large divergence between accessions 20 and 21, accession 19 continues in isolation from the others.

The eigenvalue estimates corresponding to the two first canonical variables explained 98.36% of the total variation, allowing for a satisfactory description of the genetic divergence in the accessions through the score graph dispersion in relation to the first two canonic variables (Figure 2). Once again it was verified that accessions 20 and 21 are the most divergent and all other accessions seem united in a second group. The relative contributions of the evaluated descriptors to the genetic divergence of the 21 turmeric accessions are described in Table 5. All evaluated traits contributed to the determination of the genetic divergence among the accessions, although in a very disproportional manner. The traits: NH, WH, ND, FW, and TW, summed up, contributed with only 16.52% to the evaluation of the genetic divergence among the accessions. Trait PC, for instance, contributed with only 0.82%,

indicating that this trait did not cause significant alterations in the results and could be discarded in future experiments. CC was the trait that contributed most to the genetic divergence (64.77%), followed by DW (18.71%). Together, these two descriptors accounted for 83.47 % of the total variation. The fact that CC presented a large contribution to the genetic divergence influenced all other analyses greatly, as expressed in the dendrogram constructed by the nearest neighbor method (Figure 1) in the display of the first two canonical variables (Figure 2).

The classification of the 21 accessions in five groups by the Mahalanobis distances shows that this kind of analysis was effective to evaluate the existing variability even when the accessions were very similar, as in the case of the accessions from Mara Rosa. Besides, the multivariate analyses demonstrated the large contribution of descriptors CC and DW to the divergence among accessions and groups and graded up these two descriptors even more in the evaluation of turmeric

accessions. Accession 19 stood out with the best means of the analyzed descriptors, though all other eight accessions that formed the group with the highest CC means were also considered promising for future improvement studies.

Knowledge on the genetic diversity among the accessions allows correct recommendations of cultivation and use. Besides, the morpho-agronomical characterization of genotypes associated with the genetic divergence can serve as guidelines for actions taken in improvement programs. The establishment of hybrids in *C. longa* is still a little effective system due to the sparse achievement of viable seeds (Sasikumar et al. 1999). The search for new genotypes in genebanks of the species and the establishment of somaclonal variants via tissue culture or of polyploids per protoplast fusion are some of the possible alternatives aiming at the increase of the genetic variability of the crop (Valois et al. 2001).

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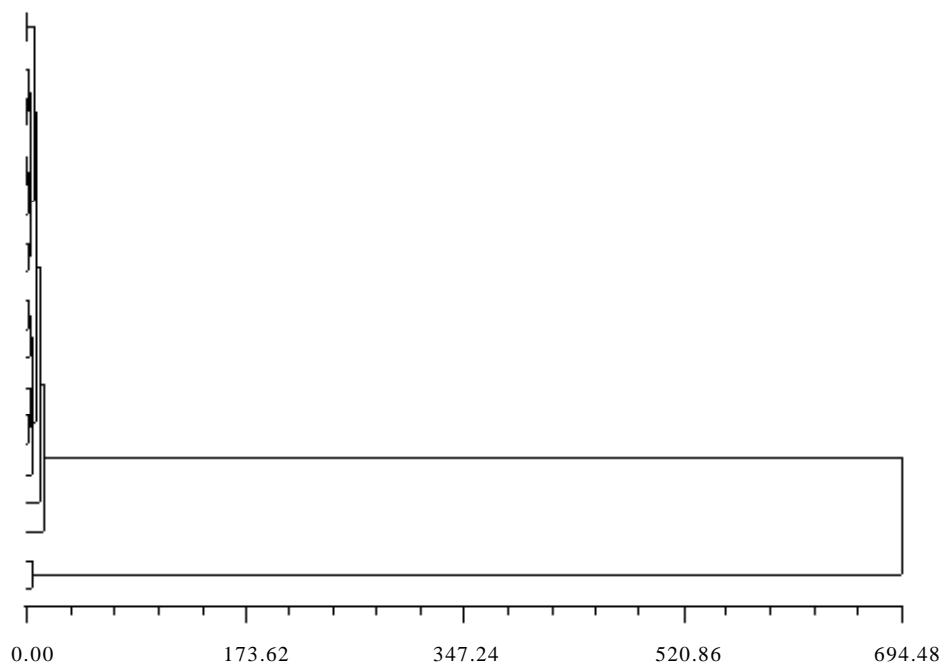


Figure 1. Single linkage dendrogram related to 21 turmeric genotypes, based on the Mahalanobis distance

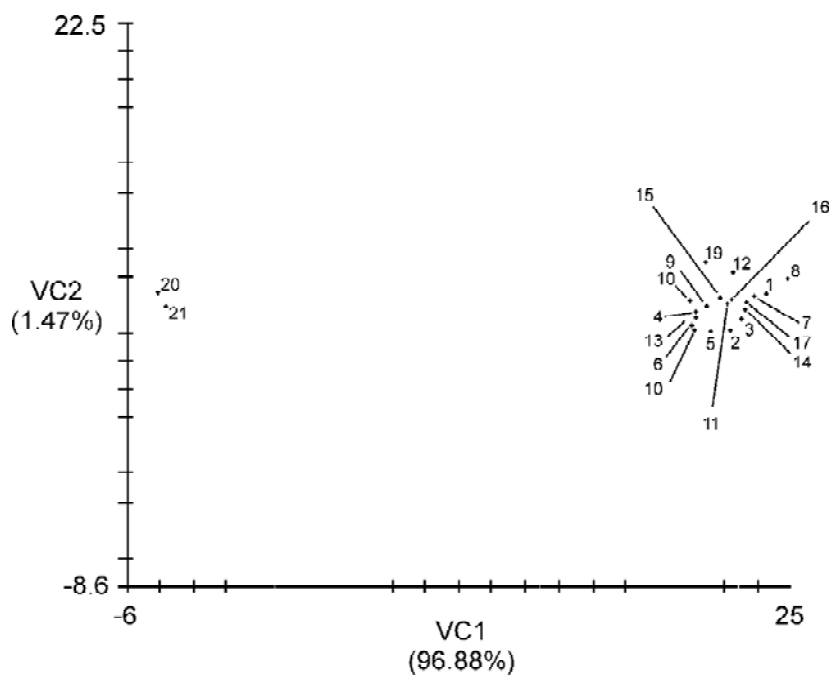


Figure 2. Display of the first two canonical variables (VC1 and VC2). Values of the variation percentages obtained for each canonical variable are indicated in brackets. Genotypes: 1 Alexânia – GO, 2 Goiânia – GO, 3 Botucatu – SP, 4 Ibitinga – SP, 5 Lavras – MG, 6 Campinas – SP (IAC⁺), 7 Goiânia – GO, 8 Mara Rosa – GO, 9 Mara Rosa – GO, 10 Rubiataba – GO, 11 Mara Rosa – GO, 12 Mara Rosa – GO, 13 Mara Rosa – GO, 14 Mara Rosa – GO, 15 Mara Rosa – GO, 16 Mara Rosa – GO, 17 Mara Rosa – GO, 18 Mara Rosa – GO, 19 Mara Rosa – GO, 20 Campinas – SP (IAC⁻), 21 Mara Rosa – GO

Divergência genética entre acessos de *Curcuma longa* L.

RESUMO - Avaliou-se a divergência genética entre 21 genótipos de açafrão com base em características morfo-agronômicas e em procedimentos multivariados, visando selecionar genótipos divergentes e mais produtivos. O experimento foi conduzido em 2001/2003 em blocos ao acaso com quatro repetições. Foram avaliados seis descritores relacionados à produção de rizomas, além do teor de curcuminóides. A partir da matriz de distância generalizada de Mahalanobis foram aplicados os algoritmos de Tocher e do vizinho mais próximo e processada análise de variáveis canônicas. Também se avaliou a importância relativa dos descritores. As técnicas multivariadas foram eficientes para o estudo da divergência genética e permitiram a separação dos 21 acessos em cinco grupos. Teor de curcuminóides e peso seco foram os descritores que mais contribuíram para a divergência genética e permitiram indicar os melhores acessos para programas de melhoramento, visando materiais produtivos e com altos teores de curcuminóides.

Palavras-chave: Açafrão, divergência genética, análise multivariada, algoritmo de Tocher.

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