Stability of Genetic Divergence among Eggplant Accesses in Three Stages of Development.

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ABSTRACT

Genetic divergence stability among 19 eggplant accesses was estimated in three stages of development: vegetative, reproductive and productive. The experiment was carried out in a randomized block design, with three replications and four plants per plot. For each developmental stage, the divergence was calculated from 12, 12 and 5 characteristics, respectively. The cluster analysis, applied to the genetic distance matrix (D^2), showed the formation of two clusters in the vegetative stage, four in the reproductive stage and six in the productive stage. Therefore, it was obseved that productive stage characteristics have greater discriminatory capacity. However, inconsistency in respect to the number and composition of clusters formed in different stages was observed. It can be concluded that the genetic divergence estimated among accesses is related only to the variability of the characteristics used in its estimation, and that extrapolation of this variability to other characters may lead to wrong interpretations.

KEY WORDS: Solanum melongena, genetic resources, breeding, multivariate analysis.

INTRODUCTION

The great interest in genetic diversity arises from the possibility of demonstrating that phenotypic mean values express, in a larger or smaller degree, the genotypic value of an individual. Thus, while evaluating the divergence among populations, based on average phenotypic values, the divergence among genotypic values associated with gene frequency in different sample units (populations, varieties, clones, etc.) is also evaluated.

According to Falconer and Mackay (1996), the magnitude of the heterosis manifested in a cross between two samples depends on the square of the gene frequency difference multiplied by the dominant deviation of the character under analysis. Thus, genetic divergence is associated with heterosis (Cress, 1966), which increases the interest in the subject.

Among the several techniques used to express divergence between samples genetic base, the Mahalanobis' generalized distance (D^2) stands out as one of the most robust (Rao, 1952). The cluster analysis based on D^2 data is used for grouping samples in such a way that a high level of homogeneity within each group and high heterogeneity between groups is obtained (Johnson and Wichern, 1982).

Despite all the interest in the subject , the interpretation of genetic divergence analysis among samples is often difficult due to the non-repeatability of the divergence values, which can vary according to the number of analyzed characters (Arunachalan, 1981; Cruz, 1994), genotype-environment interactions (Singh and Gill, 1984; Camussi et al., 1985; Naidu and Satyanarayana, 1991), and according to the variations along the years of cultivation (Dias and Kageyama, 1997). In this sense, Viana et al. (1991) found variation in composition and number of clusters formed among different cuts of sugarcane.

Aiming at evaluating genetic divergence stability among eggplant accesses estimated in different stages of plant development, the following studies were carried out: i) evaluation of the genetic divergence stability among 19 eggplant accesses in three stages of development: vegetative, reproductive and productive; ii) analysis of the existing similarity in terms of genetic divergence estimation among access clusters, in the different stages of development.

MATERIALS AND METHODS

Genetic material

Seventeen eggplant (*Solanum melongena*) accesses from the vegetable germplasm bank of ESALQ/USP (Table 1) and two hybrids commercially grown in Brazil ('Napoli' and 'Super F 100').

Field evaluation

The experiment was conducted in Piracicaba - SP, in a randomized block design, with three replications and eight plants per plot, the four central plants being selected as a sample unit. Growing conditions were maintained according to the recommendations of Filgueira (1982)

The accesses were evaluated for 29 characteristics, based on the eggplant (IBPGR, 1990) descriptor list and groupel into three sets,according to the stage of development:

Vegetative: period between germination and the begining of flowering.

Table 1 - Eggplant genotypes from the ESALQ/USP vegetable germplasm bank.

[1/]H 1: 'Nápoli'	L 9: PI 269953
H 2: 'Super F100'	L 10: PI 166995
[2/]L 1: PI 206472	L 11: 'Annamalai brinjal'
L 2: 'Long green'	L 12: 'Indiana'
L 3: 'Campineira'	L 13: PI 319855
L 4: 'Florida market'	L 14: Sel ESALQ-1
L 5: 'E 22'	L 15: Sel ESALQ 2
L 6: PI 169667	L 16: Sel ESALQ 3
L 7: PI 210026	L 17: PI 176760
L 8: PI 224690	

[1/] H designates hybrids; [2/] L designates accesses.

1) number of days to emergence (NDE);
2) average cotyledons (ACL) length (mm);
3) average cotyledons (ACW) width (mm);
4) average hypocotyl (AHL) length (mm);
5) average radicle (ARL) length (mm);
6) average stem diameter (mm) (ASD), 70 days after transplanting, measured at the fifth internode, from ground level.

The followings characteristics in this stage of development were measured 70 days after transplanting.

7) average internode (AIL) length (cm), measured at the fifth internode from ground level;
8) average leaf blade (ALBL) length (cm);
9) average leaf blade (ALBW) width (cm);

10) average petiole (APL) length (cm);
11) average number of leaves (ANL), data transformed into $\sqrt{x}$;
12) average plant (APH) height (cm).

Reproductive: period between flowering and the beginning of fructification.
1) average number of days to flower (ANDF);
2) average number of buds/inflorescence (ANBI);
3) average number of flowers/inflorescence (ANFI), transformed into $\sqrt{x}$;
4)average number of fruits/inflorescence (ANFRI),transformed into $\sqrt{x}$;
5) average number of petals (ANP);
6) average number of sepals (ANS);
7) average flower stalk (AFSL) length (cm);
8) average flower stalk (AFSD)diameter (mm);
9) flower stalk (FSW) weight (g)
10) average calyx (ACD) diameter (mm)
11) calyx (CW)weight (g);
12) average sepals (ASL) length (mm);

Productive: period of commercial harvest of fruits.
1) average commercial fruits (ACFL) length (cm);
2) average commercial fruits (ACFD) diameter (cm) at their largest diameter;
3) average commercial fruits (ACFW) weight (gram/fruit);
4) average number of commercial fruits/plant (ANCFP); and
5) total production of commercial fruits/plant (gram/plant) (PROD).

Statistical analysis

Data were tested for normality by the Kolmogorov-Smirnov Test, using the Sigma-Stat software. The experimental data were transformed for this analysis. A multivariate analysis was performed on the data using Wilk's L statistics to test the significance of the differences between vectors of treatment means, transformed into F correspondent values (Harris, 1975).

Genetic divergence among accesses (Rao, 1952) was estimated by the Mahalanobis' generalized distance (D^2), which is defined as: $D^2 = d'W^{-1}d$, where **d'** is transpose of the vector of difference among means of accesses for all **p** characters, **W** is the **p** x **p** matrix of residual variances and covariances and **d** is the vector of differences among means of accesses for all p characters. The Tocher method (Rao,1952) was used to define similarity groups. Estimation of inter and intra-cluster distance averages was performed according to Singh and Chaudary (1979). The Singh's criterion (1981), based on D^2 estimates, was used to identify within each set of characters those that contributed least to the divergence among the studied accesses.

The Spearman correlation (Steel et al., 1997) was calculated between the divergence values obtained from each pair of accesses in different stages of development. The coincidence coefficient among the 20 most divergent and most similar pairs of accesses was calculated, at the different stages of development. The GENES (Cruz, 1997) software was used in all the analyses.

RESULTS

Vegetative stage

The multivariate analysis applied to the vegetative stage characteristics based on Wilk's L statistics and transformed into the correspondent F value, showed significant differences ($P<0.01$) among the accesses.

As for the cluster analysis (Table 2), it showed that only two clusters were formed. The most divergent pair of accesses was L4 ('Florida Market') and L10 (PI 166995), with $D^2 = 166.003$, while L5 ('E-22') and L15 (Sel ESALQ 2) were the least divergent, with $D^2 = 3.207$. The characteristics that contributed least to the divergence (Table 4), according to Singh's criterion (1981) were: ARL, AIL, ANL, and APH.

Table 2 - Clustering of eggplant accesses by the Tocher method (Rao, 1952), based on the evaluation of characteristics from the vegetative, reproductive and productive stages.

Cluster	Stages		
	Vegetative	Reproductive	Productive
I	L1, L2, L3, L5, L6,L7,L8, L9, L10, L11, L12, L13, L14, L15, L16, L17, H1, H2.	L2, L5, L11, L12, L13.	L2, L12, L13.
II	L4.	L1, L3, L6, L8, L9, L10, L14, L16, H1, H2.	L1, L3, L6, L14, L15, L16, H1, H2.
III		L7, L17.	L7, L8, L9, L11, L17.
IV		L4, L15.	L5.
V			L10.
VI			L4.

Reproductive stage

Significant differences ($P<0.01$) among accesses mean values were detected by the L statistics, revealing the presence of significant character variability in this stage.

Four groups were formed (Table 2) with inter-cluster average distances (Table 3) superior to the ones obtained in the vegetative stage, showing greater characteristic discrimination capacity . With this set of characteristics, the most divergent accesses were: L4 ('Florida Market') and L10 (PI166995), with $D^2 = 412.91$, which are in agreement with the previous stage, while the least divergent were: L2 ('Long Green') and L12 ('Indiana'), with $D^2 = 6.19$.

As for the clusters formed in the two stages analyzed, only the L2 ('Long Green'), L5 ('E-22'), L11 ('Annamalai Brinjal'), L12 ('Indiana') and L13 (PI 319855) accesses were kept in the same cluster. The L4 ('Florida Market'), which was isolated from all other accesses in the cluster on the basis of its vegetative stage characteristics, was grouped into the last cluster in the reproductive stage, sharing it with access L15 (Sel ESALQ 2). It was found that the characteristics ANFI, ANFRI, ANP, AFSL and CW (Table 4) contributed least to the analysis of genetic divergence based on reproductive data.

Productive stage

Significant differences ($P<0.001$) were detected among accesses vectors means by the L statistics. Six clusters were formed (Table 2), and the inter-cluster average distances were superior to the vegetative and reproductive previous sets (Table 3), showing, therefore, greater access discriminatory capacity. The most divergent pair of accesses was L4 ('Florida Market') and L12 ('Indiana'), with $D^2 = 674.98$, and the least divergent pair was L2 ('Long Green') and L12 ('Indiana'), with $D^2 = 1.63$.

Table 3 - Intra- and inter-cluster distance (D^2) among eggplant accesses evaluated in relation to characteristics from the vegetative, reproductive and productive stages.

Cluster	I	II	III	IV	V	VI
I	**30.30**[1/]	**99.78**				
	34.44[2/]	*128.45*	*85.37*	*257.92*		
	3.45[3/]	***334.25***	***179.17***	***87.66***	***215.60***	***644.53***
II		**0**				
		44.31	*84.53*	*185.77*		
		28.78	***94.68***	***107.36***	***72.38***	***117.00***
III			*51.68*	***208.95***		
			24.20	***76.43***	***78.91***	***243.16***
IV				*71.59*		
				0	***74.96***	***330.74***
V					***0***	***301.60***
VI						***0***

[1/] Values in bold refer to the vegetative stage;
[2/] Values in italic refer to the reproductive stage;
[3/] Values in bold and italic refer to the productive stage.

According to of criterion Singh's (1981), the characters that made the lowest contribution to the divergence analysis were ACFW and PROD (Table 4).

DISCUSSION

It was observed that the accesses L2 ('Long Green'), L12 ('Indiana') and L13 (PI 319855) were always grouped into the same cluster (Table 2) regardless the plant stage, revealing the existence of a genetic affinity between them. Accesses L1 (PI 206472), L3 ('Campineira'), L6 (PI 169667), L4 (Sel ESALQ 1) and L16 (Sel ESALQ 3) were found to form clusters together with hybrids H1 ('Nápoli') and H2 ('Super F100') in the three stages (Table 2). This shows the similarity between these accesses and the hybrids commercially grown in Brazil.

The Spearman correlation, between the distances obtained among pairs of accesses (Table 5) estimated at the three stages is greater between consecutive stages (Vegetative and Reproductive = 0.30; Reproductive and Productive = 0.73) than between more distant stages (Vegetative and Productive = 0.16). Significance of these values shows the existence of a correlation between the divergence estimated in all eggplant development stages. Similar results were found by Viana (1991) for sugar cane.

The coincidence coefficient (Table 5) estimated for the 20 most and least divergent pairs of accesses at the three stages followed the same pattern showed by the Spearman correlation, that is, they were greater between consecutive stages and smaller between more distant stages. In this sense, the use of results from the reproductive stage as an indication of the productive stage (the Spearman correlation was

Table 4 - Characteristics contribution to divergence (D^2) in relative percentiles, based on the criterion of Singh (1981), in three stages of eggplant development.

Stages					
Vegetative		Reproductive		Productive	
Characteristic	Rel. Cont. (%)*	Characteristic	Rel. Cont. (%)*	Characteristic	Rel. Cont. (%)*
NDE[1]	28.55	ANDF[13]	8.18	ACFL[25]	13.94
ACL[2]	7.26	ANBI[14]	12.43	ACDF[26]	45.65
ACW[3]	9.56	ANFI[15]	2.09	ACFW[27]	6.63
AHL[4]	8.65	ANFRI[16]	1.10	ANCFP[28]	30.85
ARL[5]	1.84	ANP[17]	2.56	PROD[29]	2.92
ASD[6]	0.36	ANS[18]	8.28		
AIL[7]	5.01	AFSL[19]	0.65		
ALBL[8]	13.06	AFSD[20]	7.58		
ALBW[9]	8.95	FSW[21]	18.60		
APL[10]	10.70	ACD[22]	19.39		
ANL[11]	2.48	CW[23]	1.78		
APH[12]	3.57	ASL[24]	17.35		

* Relative contribution

[1] NDE: number of days to emergence, [2] ACL: average cotyledons length, [3] ACW: average cotyledons width, [4] ALH: average hypocotyl length, [5] ARL: average radicle length, [6] ASD: average stem diameter, [7] AIL: average internode length, [8] ALBL: average leaf blade length, [9] ALBW: average leaf blade width, [10] APL: average petiole length, [11] ANL: average number of leaves, [12] APH: average plant height, [13] ANDF: average number of days to flower, [14] ANBI: average number of buds/inflorescence, [15] ANFI: average number of flowers/inflorescence, [16] ANFRI: average number of fruits/inflorescence, [17] ANP: average number of petals, [18] ANS: average number of sepals, [19] AFSL: average flower stalk length, [20] AFSD: average flower stalk diameter, [21] FSW: flower stalk weight, [22] ACD: average calyx diameter, [23] CW: calyx weight, [24] ASL: average sepals length, [25] ACFL: average commercial fruits length, [26] ACFD: average commercial fruits diameter, [27] ACFW: Average commercial fruits weight, [28] ANCFP: average number of commercial fruits/plant, [29] PROD: total production of commercial fruits/plant.

0.73 and the coincidence coefficient was 50% for the most divergent pairs and 15% for the most similar ones) could be of practical interest, since these results can be obtained 70 days after transplanting (compared to the 150 days needed to evaluate the productive stage conveniently). However, the use of these results as indicators of the productive stage can lead to wrong interpretations in terms of similarity and divergence once data on reproductive stage grouped the accesses into four clusters only, whereas data on productive stage formed six clusters.

The analysis of the structure and composition variation in the clusters showed that many accesses were grouped into different clusters in each analyzed stage. For instance, access L10 (PI 166995), which based on vegetative data differed only from access L4 ('Florida Market') was found to be similar to hybrids H1 ('Napoli') and H2 ('Super F100') based on the reproductive data. Using the productive data, this access was grouped into a different cluster from those of hybrids. Similarly, accesses L4 ('Florida Market'), L5 ('E-22'), L7 (PI 210026), L8 (PI 224690), L9 (PI 269953), L11 ('Annamalai brinjal') and L15 (Sel ESALQ 2) were grouped into different clusters in the three different stages. This characterizes the inconsistency in the number and composition of clusters formed in the three stages. Such fact can be explained, as each stage and even each characteristics under analysis is coded by one or many specifics genes. Thus, while analyzing the genetic divergence with respect to a determined group of characteristics, the divergence among these groups of specific genes ,which can be linked or not to other groups of genes , is also being evaluated.

Therefore, it is clear that the genetic divergence estimated between pairs of accesses is related to a determined stage, or better yet, to the set of characteristics used in its estimation. Working with cacao (Theobroma cacao L.) Dias et al. (1997) obtained results inconsistency in number and composition of clusters formed by years of harvest similar to those observed in this strudy. Thus, in the case of eggplant, if the interest of plant breeders is to select accesses for a breeding program aiming at production of hybrids or transgressive segregation in advanced generations, they should work with divergent parents for characters related to those of interest to the breeder, because genetic divergence have been related to heterosis in the hybrids for the characteristics showing dominance (Falconer and Mackay, 1996). On the other hand, if the objective

Table 5 - Spearman correlation (r_s) and coincidence coefficient for the 20 most divergent and similar pairs, estimated from the results of the Vegetative (Veg.), Reproductive (Rep.), and Productive (Prod.) stages.

Variables	r_s	Divergent (%)	Similar (%)
Veg x Rep	0.30^{**}	55	20
Veg x Prod.	0.16^{*}	25	15
Rep x Prod	0.73^{**}	50	15

* $P < 0.05$; ** $P < 0.01$.

is the evaluation of a group of accesses aiming at the determination of a germplasm bank core collection (Holbrok et al., 1993), a set of characteristics from the productive stage should be used, since this stage has the greatest potential for discriminating between these accesses.

CONCLUSIONS

Genetic diversity among a group of accesses can be estimated from different sets of characteristics obtained at different stages of development. The diversity varies in relation to each considered set. Therefore, with respect to vegetative and reproductive characteristics the most divergent accesses were L4 ('Florida Market') and L10 (PI 166995), while L4 and L12 ('Indiana') were the most divergent for the productive characteristics.

Vegetative stage characteristics grouped the accesses into two clusters, reproductive stage characters into four and the productive stage ones into six clusters.

Divergence estimates were positively correlated, and the significance of this correlation decreases as the stages of development become more distant. However, the number and composition of clusters varies from one stage to another, suggesting that the estimated genetic divergence among accesses is related only to the variability existing in the characteristics used for their estimation, not allowing extrapolations to other non-analyzed characters.

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RESUMO

Estabilidade da Divergência Genética entre Acessos de Berinjela em três Estádios do Desenvolvimento

Para avaliar a estabilidade da divergência genética entre 19 acessos de berinjela, estimada em três estádios do desenvolvimento: vegetativo, reprodutivo e produtivo, foi instalado um experimento no delineamento em blocos ao acaso, com três repetições e quatro plantas úteis por parcela. Em cada estádio a divergência foi calculada a partir de 12, 12 e 5 caracteres respectivamente. Da análise de agrupamento, aplicada à matriz de distâncias de Mahalanobis (D^2), observou-se a formação de dois grupos no estádio vegetativo, quatro no reprodutivo e seis no produtivo. Verificou-se que os caracteres do estádio produtivo possuem maior capacidade de discriminação entre os genótipos estudados. Porém, observou-se inconsistência quanto ao número e composição dos grupos formados nos diferentes estádios. Conclui-se que a divergência genética estimada entre acessos é relacionada somente a variabilidade dos caracteres utilizados para a sua estimação e que extrapolações desta variabilidade para outros caracteres podem levar a interpretações errôneas.

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