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Genetic divergence of tomato subsamples

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ABSTRACT

Understanding the genetic variability of a species is crucial for the progress of a genetic breeding program and requires characterization and evaluation of germplasm. This study aimed to characterize and evaluate 101 tomato subsamples of the Salad group (fresh market) and two commercial controls, one of the Salad group (cv. Fanny) and another of the Santa Cruz group (cv. Santa Clara). Four experiments were conducted in a randomized block design with three replications and five plants per plot. The joint analysis of variance was performed and characteristics with significant complex interaction between control and experiment were excluded. Subsequently, the multicollinearity diagnostic test was carried out and characteristics that contributed to severe multicollinearity were excluded. The relative importance of each characteristics for genetic divergence was calculated by the Singh's method (Singh, 1981), and the less important ones were excluded according to Garcia (1998). Results showed large genetic divergence among the subsamples for morphological, agronomic and organoleptic characteristics, indicating potential for genetic improvement. The characteristics total soluble solids, mean number of good fruits per plant, endocarp thickness, mean mass of marketable fruit per plant, total acidity, mean number of unmarketable fruit per plant, internode diameter, internode length, main stem thickness and leaf width contributed little to the genetic divergence between the subsamples and may be excluded in future studies.

Key words: *Solanum lycopersicum*, characterization, evaluation, genetic variability.

RESUMO

Divergência genética de subamostras de tomateiro

Para o avanço de um programa de melhoramento genético é fundamental o conhecimento da variabilidade genética existente na espécie, o que demanda estudos de caracterização e avaliação do germoplasma disponível. Objetivou-se neste estudo a caracterização e avaliação de 101 subamostras de tomateiro do grupo Salada e duas testemunhas comerciais, uma do grupo Salada (cv. Fanny) e outra do grupo Santa Cruz (cv. Santa Clara). Foram realizados quatro experimentos no delineamento em blocos casualizados, com três repetições e cinco plantas por parcelas. Foram realizadas análises de variância conjunta e descartadas as características com interação significativa do tipo complexa entre testemunha e experimento. Posteriormente, foi realizado o diagnóstico de multicolinearidade e descartadas as

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características que contribuíam para níveis severos de multicolinearidade. A importância relativa de cada característica para divergência genética foi realizada pelo método de Singh (1981), e as de menor importância relativa foram descartadas conforme metodologia de Garcia (1998). Os resultados demonstram grande divergência genética entre as subamostras estudadas para as características morfológicas, agronômicas e organolépticas, indicando potencial para o melhoramento genético. As características sólidos solúveis totais, número médio de frutos bons por planta, espessura do endocarpo, massa média de frutos bons por planta, acidez total, número médio de frutos ruins por planta, diâmetro do entrenó, comprimento do entrenó, espessura do pecíolo principal e largura da folha pouco contribuíram para a divergência genética entre as subamostras, podendo ser descartadas em estudos futuros.

Palavras-chave: *Solanum lycopersicum*, caracterização, avaliação, variabilidade genética.

INTRODUCTION

Tomato breeding programs have aimed to increase the genetic diversity of their population base (Haussmann *et al*., 2004) in order to reach more productive cultivars (Marim *et al*., 2005; Guimarães *et al*., 2007) with better fruit quality (Guimarães *et al*., 2008) and other desirable cultivar traits.

The Vegetable Germplasm Bank of the Federal University of Viçosa (UFV - BGH) possesses over 850 recorded tomato subsamples, most of them of the salad group. Characterization of subsamples has been carried out for biotic and abiotic factors such as resistance to pests and diseases (Oliveira *et al*., 2009; Fiorini *et al*., 2010); assessment of production (Rodrigues *et al*., 2010); fruit quality (Caliman *et al*., 2005) agronomic characteristics (Castro *et al*., 2010).

The evaluation and characterization of subsamples result in large amount of information, including morphological, physiological, agronomic, biochemical, cytogenetic and molecular features. This information can be used in studies of genetic divergence to guide breeders in selecting potential crosses and strategies for genetic improvement of the species. These studies can also help determining the relative importance of characters for selecting those most informative for the characterization and evaluation of germplasm, knowledge on the relation between characters, and establishment of core collections that, with the smallest subsample number, can represent most of the genetic variability in the germplasm (Upadhyaya *et al*., 2006).

Studies on genetic divergence usually use multivariate techniques that, besides allowing the quantification of divergence among subsamples, also provide graphical representation of their relationship through dendrograms or scatter plots and identification of traits with the largest contribution to genetic divergence.

This study aimed to estimate the genetic divergence among 101 subsamples of tomato belonging to the Salad group and assess the relative importance of each of the characters analyzed.

MATERIALS AND METHODS

The experiments were conducted in the Vegetable Experimental Field of the Crop Science Department, Federal University of Viçosa (UFV), Viçosa - MG (20° 45’14" S and 42° 52' 53" W, 648.74 m altitude). The regional climate is classified as Cwa, according to Köppen. Tomato was cultivated in the conventional system in single rows spaced 1.50 m apart and 0.60 m between plants.

The experiments were arranged in a completely randomized block design with three replications and five plants per plot. The three plants in the center of the row were used for the statistical analysis. A total of 101 subsamples of tomato from the Vegetable Germplasm Bank of the Federal University of Viçosa (UFV - BGH) belonging to the group salad and two commercial cultivars (Table 1) were evaluated. The subsamples were divided into lots and evaluated in four experiments conducted between August 2003 and July 2007, each experiment with about 30 subsamples and controls.

Twenty-three characteristics related to plant morphology, production and fruit quality were evaluated following the recommendations of the International Plant Genetic Resources Institute (IPGRI, 1996).

The morphological characteristics were measured in leaves and internodes immediately above the third raceme of the second and third plants in the middle section of each plot. The following measurements were taken: leaf length (LL, cm); leaf width (LW, cm); main petiole thickness (MPT, μm), internodes length (IL, cm) and internode diameter (ID, μm).

Table 1. Identification of 101 tomato subsamples of the Salad group from the Vegetable Germplasm Bank of the Federal University of Viçosa and two cultivars (controls)

Subsample		Origin	Subsample		Origin	Subsample		Origin
1	4352	Pedro Afonso - GO	36	2076	University of Purdue - USA	71	2177	University of Purdue - USA
2	4546	Rio Pomba - MG	37	2077	University of Purdue - USA	72	2178	University of Purdue - USA
3	4547	Piedade do Rio Grande - MG	38	2078	University of Purdue - USA	73	2179	University of Purdue - USA
4	4577	Lavras - MG	39	2083	University of Purdue - USA	74	2180	University of Purdue - USA
5	4596	Ilha Murutu - Manaus - AM	40	2088	University of Purdue - USA	75	2181	University of Purdue - USA
6	4619	Marajó - Murucurá - AM	41	2089	University of Purdue - USA	76	2182	University of Purdue - USA
7	4686	Manaquiri - AM	42	2092	University of Purdue - USA	77	2183	University of Purdue - USA
8	2003	University of Purdue - USA	43	2095	University of Purdue - USA	78	2184	University of Purdue - USA
9	2004	University of Purdue - USA	44	2096	University of Purdue - USA	79	2185	University of Purdue - USA
10	2008	University of Purdue - USA	45	2097	University of Purdue - USA	80	2186	University of Purdue - USA
11	2011	University of Purdue - USA	46	2098	University of Purdue - USA	81	2188	University of Purdue - USA
12	2013	University of Purdue - USA	47	2100	University of Purdue - USA	82	2192	University of Purdue - USA
13	2014	University of Purdue - USA	48	2102	University of Purdue - USA	83	2194	University of Purdue - USA
14	2016	University of Purdue - USA	49	2105	University of Purdue - USA	84	2196	University of Purdue - USA
15	2017	University of Purdue - USA	50	2109	University of Purdue - USA	85	2197	University of Purdue - USA
16	2019	University of Purdue - USA	51	2111	University of Purdue - USA	86	2222	University of Purdue - USA
17	2020	University of Purdue - USA	52	2114	University of Purdue - USA	87	2223	University of Purdue - USA
18	2021	University of Purdue - USA	53	2115	University of Purdue - USA	88	2226	University of Purdue - USA
19	2026	University of Purdue - USA	54	2116	University of Purdue - USA	89	2227	University of Purdue - USA
20	2027	University of Purdue - USA	55	2117	University of Purdue - USA	90	2229	University of Purdue - USA
21	2029	University of Purdue - USA	56	2118	University of Purdue - USA	91	2230	University of Purdue - USA
22	2033	University of Purdue - USA	57	2120	University of Purdue - USA	92	2233	University of Purdue - USA
23	2035	University of Purdue - USA	58	2121	University of Purdue - USA	93	2234	University of Purdue - USA
24	2038	University of Purdue - USA	59	2124	University of Purdue - USA	94	2235	University of Purdue - USA
25	2039 Ama	University of Purdue - USA	60	2125	University of Purdue - USA	95	2236	University of Purdue - USA
26	2039 Verm	University of Purdue - USA	61	2131	University of Purdue - USA	96	2248	University of Purdue - USA
27	2041	University of Purdue - USA	62	2132	University of Purdue - USA	97	2255	University of Purdue - USA
28	2048	University of Purdue - USA	63	2133	University of Purdue - USA	98	2269	University of Purdue - USA
29	2054	University of Purdue - USA	64	2134	University of Purdue - USA	99	2273 sal	University of Purdue - USA
30	2060	University of Purdue - USA	65	2135	University of Purdue - USA	100	2274	University of Purdue - USA
31	2064	University of Purdue - USA	66	2141	University of Purdue - USA	101	2275	University of Purdue - USA
32	2069	University of Purdue - USA	67	2149	University of Purdue - USA	102	Fanny	Seminis
33	2072	University of Purdue - USA	68	2150	University of Purdue - USA	103	Stª Clara	Sakata
34	2073	University of Purdue - USA	69	2151	University of Purdue - USA			
35	2075	University of Purdue - USA	70	2153	University of Purdue - USA			

The fruit characteristics were measured in five fruits harvested from the second and third raceme of each of the three plants in the middle section of the plot. The characteristics included: fruit length (FL, cm); fruit width (FW, cm); pedicel scar width (PSW, µm); mesocarp thickness (MT, µm); endocarp thickness (ET, µm); central axis width (CAW, µm) and locule number (NL, unit).

For fruit quality assessment, the measurements were performed in three fruits per repetition. The following characteristics were measured: total acidity (TA), expressed by the hydrogen potential (pH); total soluble solids (TSS) in °Brix, measured with a portable refractometer; total *titratable* acidity (TTA) expressed as percentage of citric acid and sensory quality (SQ) was obtained by the ratio between TSS and TTA.

Fruit production was assessed by: mean number of marketable fruits per plant (NMF, fruit pl^{-1}), considering fruits free of pests and/or diseases; mean number of unmarketable fruit per plant (NUF, fruit pl^{-1}); mean mass of marketable fruit per plant (MMF, g pl^{-1}); mean mass of unmarketable fruits per plant (MUF, g pl^{-1}); mean mass of fruit per plant (MF, g pl^{-1}); mean number of fruit per plant (NF, g pl^{-1}) and mean total mass of fruit per plant (TMF, g pl^{-1}).

The data obtained for the characteristics evaluated in the subsamples were corrected for the environmental effect by subtracting the overall mean of the controls in the four experiments from the means of the controls of each experiment. To assess the genetic divergence among the subsamples, first, a joint analysis of variance was performed, as suggested by Cruz and Carneiro (2003). The characteristics that showed significant complex interaction (according the concept presented by Cruz & Castoldi, 1991) between control and experiment were excluded from the analysis of genetic divergence.

The multicollinearity diagnostic test was carried out to identify possible problems in the residual correlation matrix and eliminate some characteristics of moderate to severe multicollinearity.

The relative importance of each characteristic in genetic divergence was determined by the Singh's method (Singh, 1981) and the less important ones were excluded using the methodology proposed by Garcia (1998).

Groups of the subsamples were formed by the Tocher's optimization method, based on the Mahalanobis distance as dissimilarity measure. Analyses were performed using the Genes *statistical* software (Cruz, 2006).

RESULTS AND DISCUSSION

The occurrence of significant interaction between the controls and the experiments was assessed for MT, MMF, MMU, TMF, MF, TSS and SQ. This interaction can be represented by two components: one of simple nature and other of complex nature. The complex interaction indicates inconsistency of genotypes for a particular characteristc in different environments, hence, it is advised to be excluded (Cruz and Carneiro, 2003). In this study, only TSS showed complex interaction and was excluded.

Severe multicollinearity (Table 2) was found between NMF and NF, FW and ET, and MMF and TMF. These results indicated the possible exclusion of the variables NMF, ET and MMF because NF, FW and TMF are considered primary components of the total fruit production in the tomato salad group (Rodrigues *et al*., 2010). The exclusion of these variables is necessary as they may result in problems for the formation of the residual correlation matrix and bias the genetic distance estimates. A weak multicollinearity was found between NF and TMF.

After the exclusion of some variables due to the complex interaction between controls and experiments and others due to severe multicollinearity, we proceeded to the initial clustering of subsamples and analysis of the relative importance using the Singh's method (Singh, 1981). The highest relative importance was found for TMF and the lowest for NUF (Table 3). The analysis of the relative importance does not determine whether or not to exclude variables, it only ranks their importance. However, knowing these values allows us to improve the use of the resources available, and if there is the need for the evaluation of a smaller number of characteristics, we can avoid those that contribute little to the divergence (Suinaga *et al*., 2003).

Once the relative importance of the characteristics to genetic divergence of the subsamples was calculated, as recommended by Garcia (1998), we excluded the least important, NUF, and performed a new clustering using the Tocher's optimization method to evaluate the effect of the exclusion on group formation (Table 4).

The result of the clustering was identical to that obtained with the characteristic included, which showed that its exclusion did not influence the genetic divergence of the subsamples. The process of exclusion and clustering was repeated with other less important characteristics: ID, IL, TA, LW and MPT, and still no change was

Table 2. Multicollinearity diagnostic test according to Montgomery and Peck (1981) classification

Characteristics	Correlation (r)	Multicollinearity*
NMF and NF	0.95	Severe
FW and ET	0.94	Severe
MMF and TMF	0.93	Severe
NF and TMF	0.79	Weak

* Condition number (CN)/Level of multicollinearity
CN<100/Weak multicollinearity (not serious problem)
100<CN<1000/Moderate to strong multicollinearity
> 1000/Severe multicollinearity

observed in the clustering of the subsamples. However, at the eighth cluster analysis, when NF was excluded, the grouping of the subsamples changed and hence no more characteristics were excluded, as it became evident that new exclusions would change the genetic divergence among the genotypes.

In this study, of the 23 characteristics initially considered, only 13 (SQ, TTA, MF, TMF, NF, MUF, LN, CAW, MT, PSW, FW, FL, LL) were effectively required to analyze the genetic divergence among the subsamples, which indicates the possibility of exclusion of characteristics. The sum of the relative importances of TMF, MUF and LL was greater

Table 3. Relative importance of characteristics related to the first and last clustering after exclusion of the least important characteristics according to the Singh's method (Singh, 1981)

1st Clustering		7th Clustering	
Characteristics	**Relative importance (%)**	**Characteristics**	**Relative importance (%)**
Sensory quality	4.12	Sensory quality	4.57
Total titrable acidity	4.54	Total titrable acidity	5.08
Total acidity	1.32		
Mean fruit mass	6.54	Mean fruit mass	5.87
Total fruit mass	26.43	Total fruit mass	30.42
Total fruit number	1.76	Total fruit number	2.23
Unmarketed fruit mass	13.01	Unmarketed fruit mass	12.21
Unmarketed fruit number	0.75		
Locule number	4.76	Locule number	5.40
Central axis width	5.85	Central axis width	6.28
Mesocarp thickness	2.75	Mesocarp thickness	2.88
Pedicel scar width	3.54	Pedicel scar width	3.70
Fruit width	4.80	Fruit width	5.36
Fruit length	11.78	Fruit length	13.03
Internode diameter	1.03		
Internode length	1.05		
Main petiole thickness	1.65		
Leaf width	1.45		
Leaf length	2.77	Leaf length	2.91

Table 4. Clustering by the Tocher's optimization method of 101 subsamples and two commercial cultivars of tomato evaluated for 23 characteristics with subsequent exclusion of those with the least relative importance to the genetic divergence of the subsamples

Clustering	Groups	Subsamples
1st	I	25; 26; 18; 9; 24; 23; 14; 20; 82; 77; 36; 4; 11; 31; 85; 32; 81; 76; 71; 75; 79; 33; 67; 70; 10; 72; 86; 27; 45; 80; 66; 29; 3; 13; 42; 41; 34; 83; 69; 2; 58; 49; 57; 12; 47; 48; 28; 59; 68; 84; 52; 73; 63; 21; 35; 51; 53; 39; 56; 60; 64; 65; 15; 50; 22; 61; 102; 46; 37; 103; 19; 8; 38; 44; 16; 54; 5; 1; 91; 78; 62; 74; 40; 30; 6; 87; 7; 43; 98; 17; 55; 93; 92; 95
	II	88; 94; 101; 97; 90; 96; 100; 89
	III	99
7th	I	25; 26; 18; 9; 24; 23; 14; 20; 82; 77; 36; 4; 11; 31; 85; 32; 81; 76; 71; 75; 79; 33; 67; 70; 10; 72; 86; 27; 45; 80; 66; 29; 3; 13; 42; 41; 34; 83; 69; 2; 58; 49; 57; 12; 47; 48; 28; 59; 68; 84; 52; 73; 63; 21; 35; 51; 53; 39; 56; 60; 64; 65; 15; 50; 22; 61; 102; 46; 37; 103; 19; 8; 38; 44; 16; 54; 5; 1; 91; 78; 62; 74; 40; 30; 6; 87; 7; 43; 98; 17; 55; 93; 92; 95
	II	88; 94; 101; 97; 90; 96; 100; 89
	III	99
8th	I	25; 26; 18; 9; 24; 23; 14; 20; 82; 77; 36; 4; 11; 31; 85; 32; 81; 76; 71; 75; 79; 33; 67; 70; 10; 72; 86; 27; 45; 80; 66; 29; 3; 13; 42; 41; 34; 83; 69; 2; 58; 49; 57; 12; 47; 48; 28; 59; 68; 84; 52; 73; 63; 21; 35; 51; 53; 39; 56; 60; 64; 65; 15; 50; 22; 61; 102; 46; 37; 103; 19; 8; 38; 94; 90; 96; 44; 16; 54; 5; 1; 91; 78; 62; 74; 40; 30; 6; 87; 7; 43; 98; 17; 55; 93; 92; 95
	II	97; 101; 88; 100; 89
	III	99

than 55%, showing that they account for most of the genetic divergence between the subsamples. The Tocher's method (Table 5) clustered the subsamples into three groups: Group I, the largest group, with 92 subsamples having in general similar characteristics to the two controls, which were also clustered in this group. Groups II and III had eight and one subsamples respectively. The results show, therefore, that although the subsamples belong to the same commercial Salad group, they have genetic variability with possible gains from breeding.

Because Group I included most of the subsamples analyzed (91%) and the Tocher's method allows the estimation of intra and inter-group distances, the subgrouping procedure was carried out for this group. Six subgroups were formed with 65, 10, 5, 3, 3 and 2 subsamples, while 6 subsamples were not grouped with any other (Table 5).

The clustering of the subsamples (Table 5) was not associated with their origin (Table 1). For instance, the subsample 40 (group I) and subsample 99 (group III) from Purdue University were clustered into different groups. On the other hand, subsamples from different geographical regions of Brazil, such as the subsamples 1, 2, 3 and 4 were clustered in the same group.

Table 5. Groups and subgroups formed from 101 tomato subsamples from the VGB-UFV and two commercial cultivars by the Tocher's optimization method based on the evaluation of 13 charateristics

Groups	Subgroups	Subsamples
I	1	53; 64; 15; 58; 31; 14; 39; 35; 23; 11; 27; 9; 21; 24; 57; 33; 85; 68; 77; 36; 41; 71; 28; 82; 10; 13; 32; 4; 75; 67; 81; 76; 83; 3; 69; 29; 72; 66; 79; 19; 45; 20; 86; 70; 42; 34; 49; 80; 2; 47; 48; 59; 56; 84; 73; 12; 63; 60; 52; 51; 18; 37; 50; 65; 22
	2	25; 26; 38; 78; 74
	3	5; 6; 1; 54; 61; 43; 16; 17; 55; 62
	4	93; 98; 92
	5	44; 103; 46
	6	8; 30
	7	102
	8	7
	9	91
	10	40
	11	87
	12	95
II		88; 94; 101; 97; 90; 96; 100; 89
III		99

CONCLUSIONS

The tomato subsamples of the Salad group from the Vegetable Germplasm Bank - UFV are genetically divergent and gains can be obtained by selection.

Characteristics can be excluded without changing the original genetic divergence.

The characteristics mean total mass of fruits per plant, mean mass of unmarketable fruits per plant and fruit length are the highest contributors to genetic divergence of the tomato subsamples of the Salad group from the Vegetable Germplasm Bank - UFV .

The subsample 'BGH-2273 sal' is the most divergent among all tomato subsamples of the Salad group from the Vegetable Germplasm Bank - UFV.

Over 90% of the subsamples evaluated were clustered into the same group of the commercial controls.

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