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Diversity analysis of tomato genotypes based on morphological traits with commercial breeding significance for fresh market production in eastern USA

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Abstract

Tomato is one of the most economically important vegetable crops in the world. Objective of this study was to determine the genetic diversity of tomato based on its horticultural traits. Seventy-one tomato genotypes were planted and grown in two replications with randomized complete block design for two years. Diversity analysis produced six distinct clusters based on average-linkage method. Five principal components (PC) explained more than 92% of the phenotypic variation. Clusters produced in this analysis can be of importance for breeding programs developing specific fruit types based on consumer's demand.

Keywords: Cluster analysis; principal component analysis (PCA); tomato breeding; *Solanum lycopersicum*; *Solanum habrochaites*, *Solanum pimpinellifolium*; *Solanum lycopersicum* var. *cerasiformae.*
Abbreviations: PC_Principal component, PCA_Principal component analysis, SAS_Statistical Analysis Software.

Introduction

Cultivated tomato (*Solanum lycopersicum* L.) has been in existence for only about 400 years, introduced to Europe from the center of origin in Central and Southern America (Boswell, 1937). The first cultigens grown in the United States of America (USA) came from England and France and represent a narrow range of the genetic diversity due to bottlenecking in the cultivated tomato germplasm that occurred in Europe (Rick, 1976) and by subsequent selection. Genetic variation in wild species has been the source of traits for crop improvement in quality and disease and insect resistance in modern breeding programs (Rick and Chetelat, 1995). Intense breeding programs worldwide have resulted in tomato being the second most important vegetable in production in the world (FAOSTAT, 2014). The USA ranks second among all countries in tomato production by volume, yet is the leading importer of tomatoes based on 2010 global data. These data suggest opportunities exist to expand domestic production of tomato. Success has been achieved over time (Panthee and Gardner, 2011) but the industry, and therefore breeding programs, seek to enhance production efficiency, in part through higher yielding cultivars, to meet domestic demand along with improving the quality and health benefits of the fruit and fruit products (Bergougnoux, 2014).

Knowledge on levels of genetic diversity can be a significant aid in plant breeding for various applications (Mohammadi and Prasanna, 2003) such as analysis of genetic variability in cultivars (Cox et al., 1986; Smith, 1988), identification of diverse parental combinations for creating segregating populations with maximum genetic variability for further selection (Barrett and Kidwell, 1998) and introgression of desirable genes from diverse germplasm into the available genetic base (Thompson and Nelson, 1998). In order to develop desired tomato cultivars, it is important to catalogue the genetic diversity within the germplasm (Islam et al., 2004). Morphological traits have been used to estimate genetic diversity and cultivar development since they provide a simple way of quantifying genetic variation (Fufa et al., 2005). In our program, we seek to enhance knowledge of available genetic diversity among diverse tomato germplasm and catalogue multiple horticultural traits in order to translate this data into beneficial characteristics for the fresh market tomato industry. In North Carolina, tomato breeding program has focused on increasing fruit size, marketable yield, improving fruit quality and advancing disease resistance (Panthee and Gardner, 2011). Growth habit, shelf life, fruit smoothness and fruit firmness have also been improved in recent years (Gardner, 1990; Gardner, 2000; Gardner and Panthee, 2010). We were interested in assessing the genetic diversity of tomato germplasm using horticultural traits potentially useful for breeding program. Here, we present the results from diversity analysis on seventy-one tomato genotypes using eight different vegetative and reproductive traits.

Results

Diversity and Correlation of Vegetative and Reproductive Traits

Tomato lines representing a diverse genetic makeup with potentially beneficial traits for fresh market production were used in this study (Table S1). Select vegetative and reprodu-

Table 1. Analysis of variance (ANOVA) of traits used to compare seventy one tomato genotypes including breeding lines, heirloom selections and wild relatives

Sources of variation	Degree of freedom	Mean of squares							
		Growth	Height	Leaf type	Leaf color	Fruit size	Fruit shape	Maturity	Fruit category
Geno	70	0.691***	2.075***	0.717***	0.207***	2.228***	6.453***	0.893***	5.608***
Error	71	0.259	0.383	0.237	0.136	0.233	0.686	0.209	0.327

*** Significant at probability levels of p≤0.001

Table 2. Pearson correlation coefficients between eight traits of tomato genotypes representative of 71 diverse selections of breeding lines, heirloom selections and wild relatives

Variable	Growth habit	Height	Leaf type	Leaf color	Fruit size	Fruit shape	Maturity	Fruit category
Growth habit	1.00							
Height	0.97***	1.00						
Leaf type	0.24*	0.21	1.00					
Leaf color	0.10	0.09	0.65***	1.00				
Fruit size	0.25*	0.24*	0.49***	0.32*	1.00			
Fruit shape	0.10	0.12	0.007	-0.05	0.51***	1.00		
Maturity	-0.10	-0.04	-0.20	-0.14	-0.37*	-0.17	1.00	
Fruit category	0.16	0.11	0.42**	0.25*	0.41**	0.19	-0.61***	1.00

*, ** and *** are significant at probability <0.05, 0.01 and 0.001 levels, respectively.

Table 3. Tomato genotypes grouped into six clusters depending upon eight morphological traits based on the Average-linkage method.

Cluster	Frequency	Typical picture	Tomato genotypes
I	1		Yellow stuffer
II	2		Yellow pear, NC22L-1W(2008)
III	7		NCEBR-8, NCEBR-6, NC30P, 918-4B(2007)-9-13, 918-4B(2007)-9-12, 918-4B(2007)-9-11, 78L-1W(2008)
IV	50		Fla8233, Fla8000, Fla7600, NC1CELBR, Brandywine, Black from Tula, Orange strawberry, Aker's West Virginia, NC161L-1W(2007), 47NC2, NC109, 72E-1(96), 48BC-1R(96), 97E-3W(95), 39BC-1(96), 97E-1W(95), 48BC-1(96), 31LB-1W(95), Rutgers, 45LB-1(98), 16BC-2(94), NC123S, NC84173, NC50-7, 46BC-2R(96), NC1CS, 89E-1W(95), 38BC-1(96), NC714, NC2CELBR, 87E-1W(95), 71BC-1(95), 16BC-1(94), Stupice, Moneymaker, Cherokee purple, 48BC-3R(96), 97E-2W(95), FD502-3-Bk, 48BC-4R(96), 30LB-1W(95), Oxheart, IRAT-L3, 17BC-1(94), G357-2(2011), G357-1(2011), 74L-1W(2008), Favorite, 38BC-2R(96), 15BC-4(94)
V	8		CRA66, HI7997, HI7981, HI7998, 52LB-4(98), 52LB-3(98), 52LB-2(98), 52LB-1(98)
VI	3		PI134417, PI114490-1-1, 081-12-1X-gsms

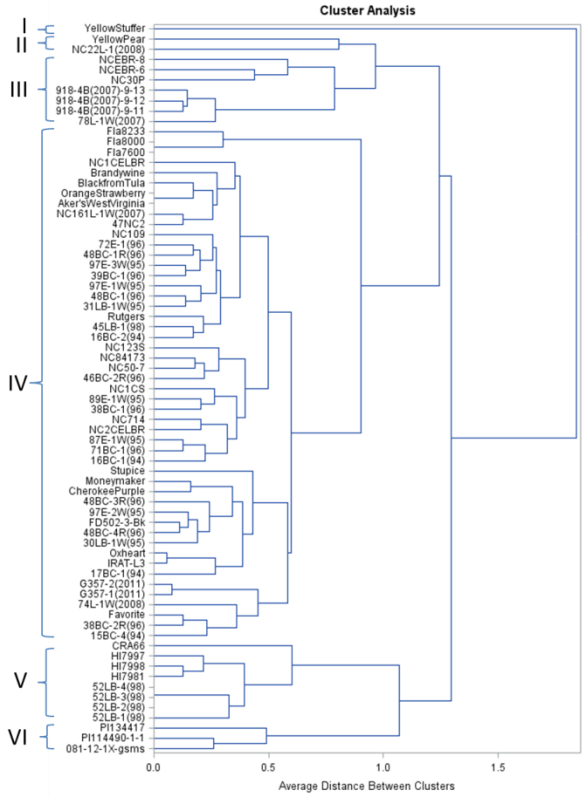

Fig 1. Dendrogram based on Average-linkage cluster analysis of tomato genotypes based upon eight phenotypic traits.

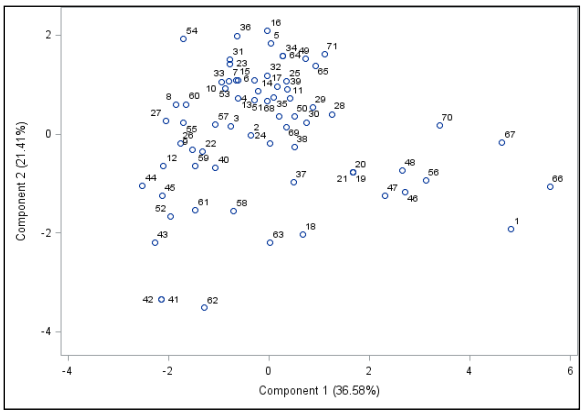

Fig 2. Component pattern based upon principal components 1 and 2 developed using eight phenotypic traits on tomato genotypes using principal component analysis.

ctive traits considered to have a direct or indirect relationship with yield were phenotyped. Analysis of variance (ANOVA) showed that all eight traits had a high level of difference among tomato lines (P<0.01) (Table 1).

Pearson correlation coefficients were calculated to assess the relationship among traits (Table 2). Significant positive correlation (r = 0.97, P< 0.01) was observed between growth type and height of tomato plants. Significant positive correlations were also observed between leaf type and several traits including leaf color (r = 0.65, P< 0.01), fruit size (r = 0.49, P< 0.01) and fruit category (r = 0.42, P = 0.01). Fruit size was significantly correlated with fruit shape (r = 0.51, P < 0.01) and negatively correlated (r = -0.37, P= 0.05) with days to maturity. Generally small-sized tomato matured earlier than larger-sized tomato. Fruit size was also positively correlated with fruit category (r = 0.41, P= 0.01). Maturity was found to be negatively correlated with fruit category (r = -0.61, P< 0.001); fruit in the cherry/grape category matured earlier than those in the plum and large-fruited categories.

Cluster analysis of 71 Tomato Genotypes

Cluster analysis using the average-linkage method grouped seventy one- tomato genotypes into six clusters (Fig 1). Tomato genotypes grouped in each cluster are presented (Table 3). Only one tomato genotype was included in cluster I consisting of an indeterminate genotype. Fruits of this genotype were large, had a unique lobed shape, which resembled bell pepper morphology, were late ripening and turned yellow when ripe. Fruits were partially hollow inside.
The second cluster included two genotypes that had an indeterminate growth habit and were tall. Fruits of these genotypes were small. Yellow pear had yellow, small, pear-shaped fruit whereas NC22L-1(2008) was a red colored grape tomato.

Table 4. Prior communality estimates, eigenvalues and cumulative proportion of variation due to eight phenotypic traits studied in seventy one tomato genotypes.

No.	Eigenvalue	Difference	Proportion	Cumulative
1	2.93	1.21	0.3658	0.3658
2	1.71	0.38	0.2141	0.5798
3	1.33	0.36	0.1665	0.7463
4	0.98	0.56	0.122	0.8683
5	0.41	0.05	0.0518	0.9201
6	0.40	0.13	0.0462	0.9663
7	0.24	0.21	0.0299	0.9962
8	0.03		0.0038	1

Table 5. Rotated factor pattern of tomato genotypes based upon eight phenotypic traits.

Trait	Factor1	Factor2	Factor3
Growth habit	9	97*	10
Plant height	6	98*	6
Leaf type	25	17	85*
Leaf color	7	3	87*
Fruit size	74*	22	28
Fruit shape	71*	13	-33
Maturity	-72*	6	-17
Fruit category	69*	1	37

Printed values are multiplied by 100 and rounded to the nearest integer. Values greater than $\pm$ 40 are flagged by an '*'.

The third cluster included seven genotypes. All of the genotypes included in this cluster had plum-shaped fruits. Leaf color of these genotypes was relatively dark green. Maturity ranged from early to late in this cluster. Growth type of these genotypes ranged from determinate to indeterminate and plant height also ranged from short to tall.

The fourth cluster included 50 genotypes of which 49 were large fruited and one was plum shaped (74L-1W(2008)) These genotypes had a standard tomato leaf type. This was a large cluster reflective of the priority in tomato breeding programs to develop large fruited tomato cultivars. This cluster contained heirloom and advanced genotypes from tomato breeding programs. These genotypes can be of importance in breeding for large fruited tomatoes.

The fifth cluster included eight genotypes. Fruits of these genotypes were medium size and round. They were mostly early maturing except CRA66. These lines ranged from semi-determinate to an indeterminate growth habit. This cluster does not appear very important for desired fruit characteristics.

The sixth cluster included three genotypes. Two of these genotypes PI114490-1-1 (*S. lycopersicum*) and PI134417 (*S. habrachaites*) are relatives of domesticated tomato. Genotypes in this cluster had an indeterminate growth habit and were tall. They had small leaves with serrated margins. Leaves of PI134417 had obvious long leaf trichomes on the leaf surface. They also had very small fruits and resembled cherry tomato. Fruits of PI134417 had significantly long fruit-hairs that did not drop even after fruits matured. Fruits of this genotype were late maturing and always stayed green.

Principal Component Analysis

Principal component analysis (PCA) revealed that there were five principal components (PC) explaining more than 92% of the total phenotypic variation among the genotypes (Table 4). Two dimensional graphical representation of component patterns based on PC1 and PC2 is shown in Fig 2. Composition of principal components indicated that the fruit traits including fruit size, fruit shape, fruit category and days to maturity were included in PC1; height and growth type in PC2 and leaf type and leaf color in PC3 (Table 5).

Discussion

Diversity in specific morphological traits targeted for their utility within a fresh market tomato breeding program was documented in this study. The 71 unique germplasms were grouped into 6 distinct clusters and these clusters provide a framework for trait selection and advancing tomato lines for specific market niches and for improving yield efficiency. Using fruit traits as an example, Cluster I had uniquely lobed fruit, similar to a bell pepper, and this genotype could be used to develop attractively shaped fruit for niche markets. In contrast, Cluster II had small fruit potentially well suited for the fresh market salad industry. Such fruit typically have high sugar content compared to large fruited tomatoes with an observed negative correlation between fruit size and soluble solids (Panthee et al., 2012; Panthee et al., 2013). Cluster III comprised genotypes that can be used in breeding plum-shaped tomatoes whereas Cluster IV represented selections comprising large and round fruit, typical for the most popular tomatoes grown in the eastern USA. The majority of selections evaluated in this study (n=50) clustered into Group IV indicative of the historical priority to develop large-fruited and round tomatoes. Cluster V and VI did not have specific fruit traits subject to obvious selection but represent lines that may confer bacterial resistance or other beneficial traits (Scott et al., 1995; Somodi et al., 1994).

This study demonstrated a high level of morphological diversity within the 71 germplasms evaluated. Documentation of morphological traits is informative for breeding programs since high levels of diversity based on morphological traits, including traits of commercial value, are associated with tomato lines that have a low level of genetic diversity when assessed using molecular markers (Cebolla-Cornejo et al., 2013; Mazzucato et al., 2008). In the present study, unlike some of the past studies (Cebolla-Cornejo et al., 2013; Corrado et al., 2013; Hu et al., 2012; Yi et al., 2008; Zhou et al., 2015), we did not use any molecular technique to characterize the germplasm. Morphological traits have been utilized for similar studies in the past in tomato. For example, 97 tomato accession from Iran and Turkey were characterized and grouped into five clusters (Henareh et al., 2015). Principal component analysis found

three major PCs explained 71% of the total phenotypic variation with leaf type and days to maturity as the major traits determining the clustering of the tomato genotypes, similar to the results in this study. Likewise, Mazzucato et al. (2008) characterized 61 tomato genotypes from Italy using molecular and phenotypic traits. In agreement with the present findings, fruit shape was an important component in determining groupings of the genotypes. A parallel study evaluated 67 tomato genotypes from Argentina by morphological traits and molecular markers and these clustered into three groups (Hu et al., 2012). Fruit shape was also found to be a major trait determining the genetic variation in the Argentina collection. Zhou et al. (2015) characterized 50 wild and cultivated tomato genotypes using morphological and molecular markers and found six clusters. Three principal components explained 78.5% of the phenotypic variation. Major traits in the PC were related to leaf traits, also an important set of traits found in our study. Cebolla-Cornejo et al. (2013) also performed diversity analysis on 75 tomato lines, primarily landraces from Spain, using phenotypic and molecular markers. In their study, the first PC was associated with fruit size traits whereas the second PC was associated with traits related to fruit shape; similar to our observations.

The eight traits used in this study were selected based on previous studies as highlighted and validated above. These traits represent a subset of traits that group tomato genotypes into diverse clusters and these clusters are informative for developing strategies in a fresh market tomato breeding program.

Materials and Methods

Plant materials

Seventy one genotypes including advanced breeding lines, heirlooms and wild genotypes (Table S1) were sown in flat bed metal trays in a standard seeding mix (2:2:1 (v/v/v) peat moss:pine bark:vermiculite with macro- and micro-nutrients (Van Wingerden International Inc., Mills River, NC) on May, 2013. After 10 days, seedlings were transplanted to 72-cell flats (56 cm × 28cm). After four weeks these plants were transplanted to the field at the Mountain Horticultural Crops Research and Extension Center, Mills River, North Carolina. Plots consisted of six plants with plant to plant spacing of 45 cm and 150 cm distance between rows in two replications in a randomized complete block design. Management practices for fertilization, insect management and management of foliar diseases were done according to standard recommendations (Ivors and Louws, 2013).

Data collection

Eight vegetative and reproductive traits of tomato lines were measured according to the tomato descriptor parameters published by the International Plant Genetic Resources Institute (IPGRI, 1996) with some modifications. Traits measured were growth type (1=determinate, 2=semi-indeterminate, and 3=indeterminate), plant height (cm), leaf type (1=dwarf, 2=potato leaf type, 3=standard, 4=peruvianum, 5=pimpinellifolium, 6=hirsutum, and 7=others), leaf color (1=light green, 2=green, and 3= dark green), fruit shape (1= Flattened (oblate), 2= slightly flattened, 3= rounded, 4= highly rounded, 5= heart-shaped, 6= cylindrical (long oblong), 7= pyriform, 8= ellipsoid (plum-shaped), and 9= others), fruit size (1= very small, 2= small, 3= intermediate, 4= large, and 5= very large), days to 50% maturity (days) and fruit category (1=cherry/grape, 2=plum or 3=large-fruited). All traits were measured by visual observations.

Statistical analysis

Data analysis was conducted using SAS Software version 9.3 (SAS Institute Inc, 2011). Analysis of variance (ANOVA) was done to identify if genotypes were significantly different from each other for the traits. Correlation analysis was performed using the Pearson product-moment correlation coefficient (Puth et al., 2015). Principal component analysis (PCA) and cluster analysis was performed using chord distance coefficient and the average-linkage method on the data sets as described by Mazzucato et al. (2008).

Conclusion

Tomato genotypes were characterized into various clusters based on morphological traits in the present study, which has direct relevance in breeding strategies. Morphological traits including fruit shape and size, growth habit, and days to maturity were useful to cluster the genotypes into various groups. These are also the traits with economic relevance in breeding programs. Although molecular characterization was lacking in the present study, which can be planned in the future similar study to explore further detailed characterization of the tomato germplasm, information from the present study can be utilized in planning future breeding strategies.

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