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Determination of genetic diversity in strawberry (*Fragaria* × *ananassa*) using principal component analysis (PCA) and single linkage cluster analysis (SLCA)

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To assess the nature and magnitude of variability in 22 genotypes, strawberry from diverse eco-geographic origins were evaluated using Principal Component Analysis (PCA) and single linkage cluster analysis (SLCA), assessing the divergence and similarity. The experiment was laid out in randomized complete block design (RCBD) with three replications. The genotypes were classified into five for the determination of variability and four cluster groups for similarity by PCA and SLCA, respectively. The highest inter cluster distance was observed between cluster II and V (129.39), followed by IV and V (114.082) and the lowest between II and IV followed by III and IV. The highest intra- cluster distance was observed for cluster III and the lowest for the cluster VI and V. PCA showed that four of the four principal component axes had Eigen values greater than one and altogether accounted for 77.34% of the total variation. The first two accounted for 57.88% with PCA 1 accounting for 36.31% and PCA 2 accounting for 21.57%. The major contributing traits in PC1 was number of flowers, number of leaves and number of fruit/plant of leaflets per plant; whereas in PC2, fruit length and fruit weight were major contributors for higher yield and quality. Thus, PCA was useful tool for identifying the characters responsible for major variability and to be used for breeding programme for higher yield and yield attributing traits, whereas SCLA proved to be a better tool in multivariate analysis since it provided much clearing information concerning the extent of relationship among the genotypes.

Key words: Strawberry (*Fragaria* × *ananassa*), genetic diversity, principal component analysis, single linkage cluster analysis.

INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch.), an important family member of *Rosaceae*, is one of the most popular soft fruit in the world. The strawberry fruits are of a very delicious taste and fresh aroma. The genus *Fragaria* consists of approximately 20 species, with a base chromosome number of x = 7. The ploidy levels of natural wild strawberry species includes diploid (2n = 2x = 14), tetraploid (2n = 4x = 28), pentaploid hexaploid,

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octaploid and nonaploid plants (Hammer et al., 2008). The cultivated strawberry is an octoploid (2n = 8x = 56) stoloniferous perennial herb (Debnath and Teixeira da Silva, 2007). It has a wide range of climatic adaptation which includes Mediterranean, temperate, subtropical and taiga zones (Hancock et al., 1991). The level of genetic diversity in strawberry germplasm is a critical consideration in breeding new strawberry cultivars. Inbreeding in cultivated strawberry leads to rapid loss of vigour, yield and fruit size (Morrow and Darrow, 1952; Spangelo et al., 1971). Narrowing of the germplasm is based on results from intense selection for certain traits and the continued reliance on a relatively small parent pool consisting of proven cultivars and advanced selections (Sjulin and Dale, 1987). Increasing genetic diversity through the introduction of divergent cultivated and wild *Fragaria* germplasm has been acknowledged as an important means for preventing inbreeding depression (Luby et al., 1991; Sjulin and Dale, 1987).

Determining the amount of genetic diversity available in germplasm has typically been estimated through pedigree analysis (Noiton and Alspach, 1996; Scorza et al., 1985; Sjulin and Dale, 1987). To improve the yield through selection, information on nature and magnitude of variability present in a population is an important prerequisite for starting any breeding programme. For a successful breeding program, the presence of genetic diversity and variability play a vital role to meet the diversified goals of plant breeding such as breeding for increasing yield, wider climatic adaptation, desirable quality, pest and disease resistance, etc. Selection of genetically diverse parents in any breeding programme is of immense importance for successful recombination breeding (Arunachalam, 1981). A sound understanding of genetic variability of different targeted traits is a useful tool in genetic improvement of the crops. Genetic divergence analysis estimates the extent of diversity existed among selected genotypes (Mondal, 2003). Precise information on the nature and degree of genetic diversity helps a plant breeder in choosing the diverse parents for purposeful hybridization (Samsuddin, 1985). Improvement in yield and quality is normally achieved by selecting genotypes with desirable character combinations existing in the nature or by hybridization. Numerical taxonomic techniques have also been successively used by many workers to classify variation patterns at both intra and inter-specific levels (Sneath and Sokal, 1973; Ariyo and Odulaja, 1991).

The D^2 statistic provides a quantitative measure of genetic divergence among populations and assists in classifying genetic stocks into distinct groups which is helpful for further evolving superior genotypes. Breeding for a particular set of growing conditions, it is highly important to know the use of local populations, since in them, the relationships among yield components are balanced and in harmony with the effects of the specific climatic and edaphic factors. Principal Component Analysis (PCA) is a descriptive method which shows the pattern of co-variation of characters among the individuals (Rhodes and Martin, 1972). It tends to reduce the dimension of multivariate data by removing inter-correlation among variables and allows a multi-dimensional relationship to be plotted on two or three principal axes (Hayman, 1967). The relative discriminating power of the axes and their associated characters are measured by Eigen-values and component scores, respectively. However, PCA alone would not give an adequate character representation in terms of relative importance when numerous characters are considered simultaneously (Shalini et al., 2003). To complement the results of such multivariate analysis, metroglyph analysis and single linkage cluster analysis (SLCA) are often employed to classify the variation. SLCA is an agglomerative technique which shows the pattern of relationship between individuals of a population (Ariyo and Odulaja, 1991). SLCA is generally employed to summarize the position of accessions by sorting them into distinct groups. It is often used to illustrate patterns of co-variation of characters among individuals.

Thus, this study was aimed at identifying the major characters responsible for variation among strawberry genotypes with a view to grouping accessions and identifies potential parental stocks within groups employing the combined technique of PCA and SLCA.

MATERIALS AND METHODS

Experimental design

The present research study was carried out at the research farm of Central Institute of Temperate Horticulture (CITH), Srinagar, for two years that is, year 2010 and 2011. The experimental farm is situated at latitude of 34° 05 N and longitude of 74° 50 E at an altitude of 1640 m above the sea level. The 22 strawberry genotypes were sourced from different parts of India, and were used for this study (Table 1). A complete randomized block design (CRBD) was adopted. Each experimental plot was 4.5 m^2. The distance between strawberry transplants was 45 × 45 cm within inter and intra rows. The planting date was on 15th October, 2010 and 2011. Commercially ripe fresh fruits were harvested in first week of May from randomly selected to represent the population of the plantation. The average maximum temperature of 19.63°C and minimum of 6.52°C, the amount of rainfall of 60.72 mm, relative humidity of 58.35%, evaporation of 2.45 and soil characteristics namely, pH = 6.81, EC = 0.36 dSm^{-1} were recorded in growing season from the year 2010 to 2011.

Character measurement

A total of 14 qualitative and quantitative traits representing vegetative plant characteristics and characters relating to the yield and vigour of the plants were measured quantitatively over the course

Table 1. Strawberry genotypes used in study, their Source and respective Code.

Genotype	Code	Source	Genotypes	Code	Source	Genotype	Code	Source
Katrain Sweet	1	NBPGR, India	Fiana	9	NBPGR, India	Black More	17	NBPGR, India
Dil Pasand	2	NBPGR, India	Banglora	10	NBPGR, India	Heera	18	NBPGR, India
Red Cross	3	NBPGR, India	Douglus	11	NBPGR, India	Chandler	19	NBPGR, India
Larson	4	NBPGR, India	Senga Sengana	12	NBPGR, India	Brighten	20	NBPGR, India
Camma Rosa	5	NBPGR, India	Majestic	13	NBPGR, India	Howard	21	NBPGR, India
Elasta	6	NBPGR, India	Phenomenal	14	NBPGR, India	Missionary	22	NBPGR, India
Anthea	7	NBPGR, India	EC-22355	15	NBPGR, India			
EC-102642	8	NBPGR, India	Shastha	16	NBPGR, India			

NBPGR (National Bureau of plant genetic resources, New Delhi, India).

of one growing season. Qualitative assessments of the plants were done using at least 20 fruits of each genotype. A range of morphological traits was recorded including vegetative, floral and fruit characteristics and any other unusual characteristics also observed. Early yield (the first three harvest times) and total yield were measured calculating amount at each picking. Genotypes were individually analyzed for physical characteristics. Fruits were weighted in the air on a Sartorius balance of accuracy of 0.001 g. Fruit volume was calculated by a liquid displacement method. The length and diameter of the fruit were measured with a Mitutoyo digital vernier caliper. The measurement of fruit length was made on the polar axis, that is, between the apex and the end of stem. The maximum width of the fruit, as measured in the direction perpendicular to the polar axis, is defined as the diameter. The fruits obtained from each treatment were pooled, and mixed with a laboratory mixer then filtered through Whatman's No. 1 filter paper to obtain a clear juice. Total soluble solids (TSS; °brix) was determined by digital hand held refractometer (ATAGO-Japan), using two drops of filtered fruit juice in replicates TA was determined by using 10 g aliquots of strawberry fruits poured in 50 ml of distilled water and titrated with 0.1N NaOH to an end-point of pH 8.1.

TA was expressed as percentage of citric acid and was calculated using the method reported by Han et al 2004; and Ruck, 1963.

Data analysis

Data collected on the quantitative characters were analyzed using SAS Microsoft windows 9.2 (SAS Institute, 2011), employing the method outlined by Steel and Torrie (1980). Genetic diversity was studied following Mahalanobis (1936) generalized distance (D^2) extended by Rao (1952). Average intra-cluster distance was calculated by the following formula as suggested by Singh and Chaudhury (1985). Average intracluster:

$$D^2 = \frac{\Sigma D^2 i}{n}$$

Where, ΣD^2_i = Sum of distances between all possible combination (n) of the varieties/lines included in a cluster; n = all possible combinations to assess genotypes position in distinct group.

PCA and SLCA were used for the determination of genetic variation and percentage similarity within genotypes. The PCA produced Eigen-vectors and principal components scores that were used respectively to measure the relative discriminative power of the axes and their associated characters. The cluster procedure was used to produce distinct groups of the 22 genotypes on the basis of the genetic relationship while using the character variation. SLCA summarized the position of accessions into a dendrogram at intervals of 5% level of similarity, starting from 100 to 66% level of similarity, when all the 22 genotypes occurred in a single cluster.

RESULTS

Data on variability parameters is presented in Table 2 and the variability of each trait was expressed by standard deviation and the coefficient of variation. The lowest values of standard deviation were recorded in the cases of the titrable acidity and the TSS °brix while it was highest for fruit yield leaflets per plant. The coefficient of variation was lowest for the fruit set percentage followed by TSS while highest coefficient variation was found for yield and titrable acidity average fruit weight and number of flower/plant. Morphological and yield analysis based on different characters showed high polymorphism with 22 strawberry genotypes. The D^2 value estimates of genetic divergence for the strawberry genotypes suggested the resolution of 22 strawberry genotypes into five distinct clusters (Table 3) with wide range of diversity in the experimental material for majority of characters including the chemical traits (Figure 1). Cluster III consisted of a maximum of 13 genotypes (59.09%), cluster I consisted 4 genotypes (18.18%), cluster II consisted 3 genotypes (13.63%) and cluster IV and V consisted only 1 genotypes (4.54 and 4.54%), respectively. Intra and inter cluster distances are presented in Table 3. The highest inter cluster distance was observed between cluster II and V (129.39) followed by IV and V (114.08), and the lowest between II and IV followed by III and IV. The highest intra- cluster distance was observed for the cluster III and the lowest for the cluster VI and V. The highest values of inter-cluster distance indicated that the accessions' belonging to cluster III was far way from those of cluster V.

Table 2. Variability of quantitative traits in 22 strawberry genotypes.

Variable	Minimum	Maximum	Mean	Standard deviation	CV%
Flower truss/plant	3.33	9.00	5.74	1.32	22.95
Flowers/plant	27.33	86.00	52.17	14.09	27.01
Crowns/plant	3.66	7.66	5.27	1.15	21.78
Leaves/plant	22.66	57.33	36.67	8.78	23.95
Leaflets/plant	68.00	172.00	108.36	27.34	25.23
Fruit set (%)	91.66	98.38	95.62	2.18	2.28
Fruit weight (g)	5.10	14.41	9.32	2.58	27.67
Fruit length (mm)	21.80	42.20	30.66	6.13	20.01
Fruit breadth (mm)	18.68	31.85	25.79	3.82	14.80
Number of fruits/plant	26.78	83.79	49.83	13.37	26.82
TSS (^{0}Brix)	6.06	10.50	8.54	1.19	14.04
Acidity (%)	0.30	0.90	0.54	0.16	29.64
Fruit volume (ml)	7.00	15.00	10.48	2.37	22.62
Yield(q/ha)	13.08	45.33	28.45	9.93	34.89

Table 3. Average intra- (bold face) and inter-cluster distance (D^2) of 22 strawberry genotypes.

	1	2	3	4	5
1	12.90	83.24	45.03	68.01	49.09
2		11.37	41.81	30.46	129.29
3			20.84	36.62	90.39
4				0.00	114.08
5					0.00

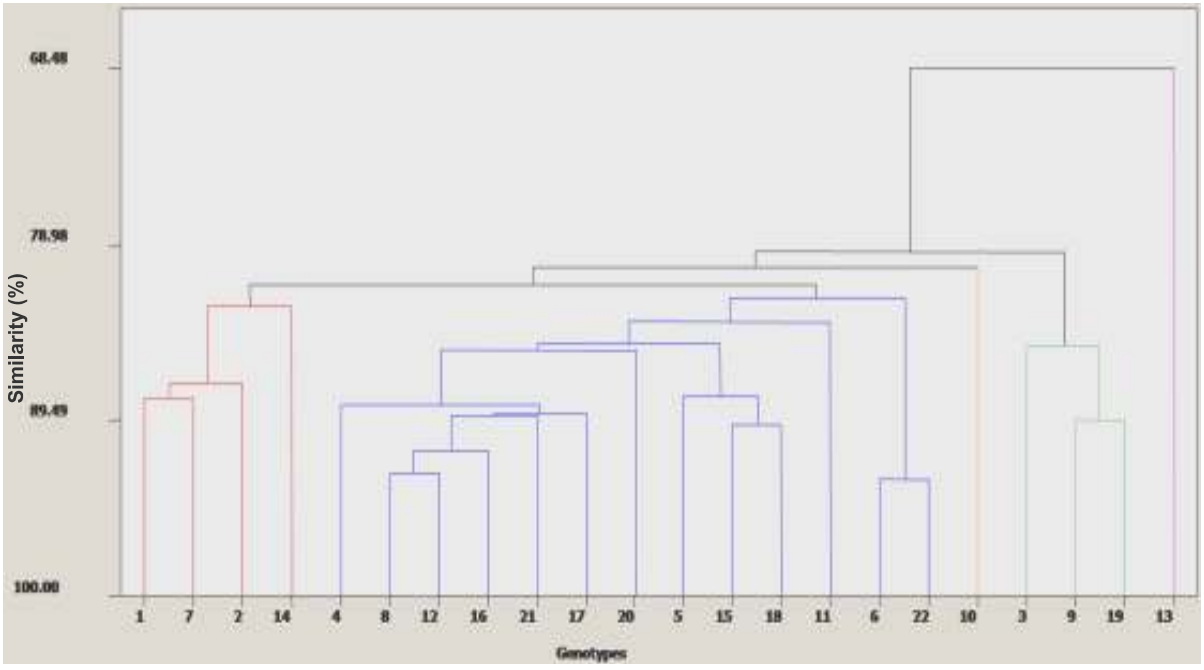

Figure 1. Dendrogram SLCA of the 22 strawberry genotypes.

Table 4. Cluster means for fourteen yield and yield contributing characters in Strawberry genotypes.

Character	I (1, 2, 7, 14) (18.8%)	II (3, 9, 19) (13.39%)	III (21, 8, 12, 17, 20, 5, 15, 18, 11, 6, 16, 22, 4) (59.09%)	IV (10) (4.54%)	V (13) (4.54%)
Flower truss/plant	6.24	4.77	5.74	3.33	9.00
Flowers/plant	62.66	34.16	52.40	27.33	86.00
Crowns/ plant	6.66	4.44	4.92	5.00	7.00
Leaves/plant	46.91	27.72	34.29	32.66	57.33
Leaflets/plant	140.75	70.66	103.00	98.00	172.00
Fruit set (%)	95.17	96.28	95.29	98.00	97.43
Fruit weight (g)	9.69	8.53	9.59	10.35	5.66
Fruit length (mm)	39.38	28.76	28.50	25.28	34.89
Fruit breadth (mm)	23.62	24.89	26.88	30.25	18.68
Number of fruits/plant	59.63	33.21	49.81	26.78	83.79
TSS (^{0}Brix)	8.94	9.02	8.21	10.33	7.96
Acidity (%)	0.40	0.60	0.554	0.60	0.60
Fruit volume (ml)	10.91	10.11	10.71	9.33	8.00
Yield (q/ha)	34.66	30.77	24.22	34.83	45.33

Table 5. Principal component analysis of the Strawberry showing the principal component cores, Eigen values and percentage total variance accounted for by the first four principal component axes.

Character	PRIN1	PRIN2	PRIN3	PRIN4
Flower truss/plant	0.285	0.225	-0.342	0.297
Flowers/plant	0.387	-0.024	-0.229	0.159
Crowns/plant	0.272	0.300	0.068	-0.128
Leaves/plant	0.415	-0.018	0.138	-0.062
Leaflets/plant	0.408	0.043	0.108	-0.086
Fruit set (%)	-0.075	0.080	0.591	0.207
Fruit weight (g)	-0.184	0.493	-0.112	0.069
Fruit length (mm)	0.181	0.361	0.323	-0.113
Fruit breadth (mm)	-0.302	0.301	-0.189	0.211
Number of fruits/plant	0.387	-0.015	-0.181	0.175
TSS (°Brix)	-0.054	-0.173	0.360	0.356
Acidity (%)	-0.072	-0.216	-0.176	0.620
Fruit volume (ml)	-0.139	0.511	-0.094	0.001
Yield (q/ha)	0.126	0.221	0.302	0.460
Eigen value	5.083	3.019	1.395	1.329
%Variance	0.363	0.215	0.099	0.095
Cumulative percentage variance	0.363	0.578	0.678	0.773

The minimum inter-cluster divergence was observed between IV and V indicating that the genotype of these cluster were genetically close. The dendrogram drawn from the SLCA shows the relationship between the 22 genotypes (Figure 1). All the genotypes were distinct from each other at 100% level of similarity and had formed one single cluster at 32% level of similarity. At about 82% of similarity, genotypes 8, 12, 16, 21 and 17 had joined with genotype 4 and formed one single cluster; whereas, at 79% of similarity level, all the genotype had form a single cluster except to genotype 13 which was still distinct from other cluster. Based on the cluster means (Table 4), the important cluster was V for flower truss per plant, flower per plant, crown per plant, leaves per plant, leaflets per plant, number of fruits per plant and yield; Cluster I for fruit volume; Cluster IV for fruit set percentage, TSS, and titrable acidity and cluster IV for juice content. From the results, it is suggested that for highest number of flower truss per plant, flower per plant, crown per plant, leaves per plant, leaflets per plant, number of fruits per plant and yield from cluster V genotypes and for fruit volume from Cluster I genotypes; For fruit set percentage, TSS and titrable acidity from Cluster IV genotypes and for juice content from cluster IV genotypes could be selected as parents for hybridization program. To assess overall diversity patterns among the genotypes, the PC analysis were conducted (Table 5). Using 14 quantitative variables evaluated in the experi-

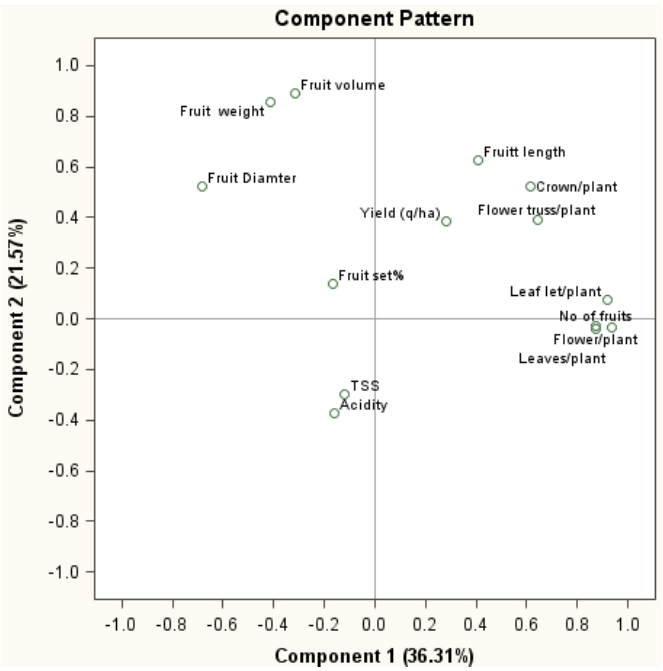

Figure 2. Configuration of the 14 qualitative and quantitative of 22 strawberry genotypes under principal component axes 1 and 2.

ments, the result of the PCA showed that four of the 14 principal component axes had Eigen values greater than one and altogether accounted for 77.34% of the total variation. The first two accounted for 57.88% with PCA 1 accounting for 36.31% and PCA 2 accounting for 21.57%. The PCA 1 was loaded with number of flower per plant (0.361), leaves per plant (0.415), leaflets per plant (0.408), PCA2 was loaded with average fruit weight (0.493) number, crown per plant (0.299), fruit length (0.361) fruit breadth and fruit volume; whereas PCA 3 and PCA 4 were loaded with percent fruit set (0.591) and TSS (0.359) flower trusses per plant, yield and titrable acidity.

Some highly significant Pearson correlations were observed between characters measured on different morphological characters and fruits parts of the strawberry (Table 6); Flower per plant, leaflets per plant and number of fruits per plant. Fruit weight positively correlated fruit breadth and fruit volume. Regularity of shapes was proved by positive correlations between weight and volume of fruits between fruit diameter and fruit volume.

DISCUSSION

Higher standard deviation and coefficient variation for yield showed high degree of diversity among the evaluated genotypes. The higher inter-cluster distances than average intra-cluster distances, confirms wide genetic diversity among the genotypes of different groups than those of same cluster. This finding was similar with the findings of Uddin and Mitra (1994). They obtained higher inter-cluster distances than the intracluster distance in multivariate analysis in sesame. The maxi-mum inter cluster distance between II and V, and IV and V suggest that if they were chosen for hybridization pro-gram to improve the vigour and yield, may give highheterotic F_1s and broad spectrum of variability in segregating generations. This result supports the findings of Samal and Jagadeb (1996) and Ahmed et al. (2002). Divergence were given greater emphasis for deciding the type of cluster for the purpose of further selection and the choice of parents for hybridization (Jagadeb and Samal, 1991). Higher inter and intra-cluster distances indicate higher genetic variability among accessions between and within clusters, respectively. The minimum inter and intra-cluster distance indicates the close genetic relationship among the accessions of two clusters and within the clusters. Accessions among the cluster separated by high D^2 values could be used in hybridization program for obtaining wide spectrum of variations among the segregates (Chahal and Gosal, 2002).

It is revealed that crosses should be made between accessions belonging to the distant clusters for high heterotic response. The relative discriminating power of the PCA was as revealed by the Eigen values which was high in PCA 1 (5.08) and lower in PCA 4 (1.32) (Table 5). Figure 2 to 4 showed projection of the agromorphological and fruit quality traits defined by principal components 1 and 2, respectively. This observation suggests that these characters are major accounting for most of the variations in strawberry and further contributing more to fruit yield in the evaluated 22 genotypes. This agrees partly with result of Nwangburuka (2011) and Ariyo and Odulaja (1991) on their works with okra. Therefore, selections from any cluster group for fruit yield must take into consideration these traits. This corroborates the report of Aremu et al. (2007) on their work with cowpea and Olika et al. (2011) working with coffee. Furthermore, the dendrogram generated from similarity or genetic distance matrices has provided an overall pattern of variations as well as degree of relatedness among accessions. Genotypes 9, 19, 13 and 10 which appears to be the most diverse may be useful as source for variable characters in strawberry improvement among the genotypes studied been the most distant. From this study, a combination of PCA and SCLA will produce better results when considering genetic variability among strawberry. This agrees with the finding of Aremu et al. (2007). However, SCLA proved to be a better tool in multivariate analysis since it provided much clearing information concerning the extent of relationship among the genotypes. This also agrees with earlier reports for cowpea and yam (Onyilagha, 1980; Aremu et al., 2007). However, these analyses are very useful for its collection, management

Table 6. Correlation matrix among 14 morphological and yield traits characteristics studied.

Character	Flower truss/plant	Flower per plant	Crown per plant	Leaves/ plant	Leaflet per plant	Fruit set (%)	Fruit weight (g)	Fruit length (mm)	Fruit breadth (mm)	Number of fruits	TSS (°Brix)	Titrable acidity (%)	Fruit volume (ml)	Yield (q/ha)
Flower truss/plant	1.000	0.637	0.475	0.526	0.528	-.225	0.100	0.334	-0.096	0.632	-.246	0.084	0.197	0.342
Flowers/plant		1.000	0.404	0.691	0.738**	-.214	-0.318	0.172	-0.464	0.995**	-0.095	-0.036	-0.255	0.188
Crowns/plant			1.000	0.592	0.583	-.059	0.175	0.536	-0.210	0.411	-0.207	-0.317	0.224	0.310
Leaves/plant				1.000	0.956**	-.098	-.419	0.441	-0.672	0.693	-0.020	-0.161	-0.342	0.213
Leaflets/plant					1.000	-.078	-.307	0.428	-0.539	0.741**	-0.052	-0.302	-0.234	0.233
Fruit set (%)						1.000	0.103	0.159	0.108	-0.129	0.110	0.027	0.115	0.222
Fruit weight (g)							1.000	0.301	0.831**	-0.311	-0.157	-0.176	0.874**	0.152
Fruit length (mm)								1.000	-0.158	0.190	-0.128	-0.328	0.396	0.357
Fruit breadth (mm)									1.000	-.466	0.053	0.040	0.674	-.009
Number of fruits/plant										1.000	-0.088	-0.039	-0.246	0.207
TSS (^{0}Brix)											1.00000	0.185	-0.217	0.078
Acidity (%)												1.00000	-0.259	0.021
Fruit volume (ml)													1.000	0.150
Yield(q/ha)														1.000

#Pearson correlation coefficients, N = 22. Prob > |r| under H0: Rho = 0.

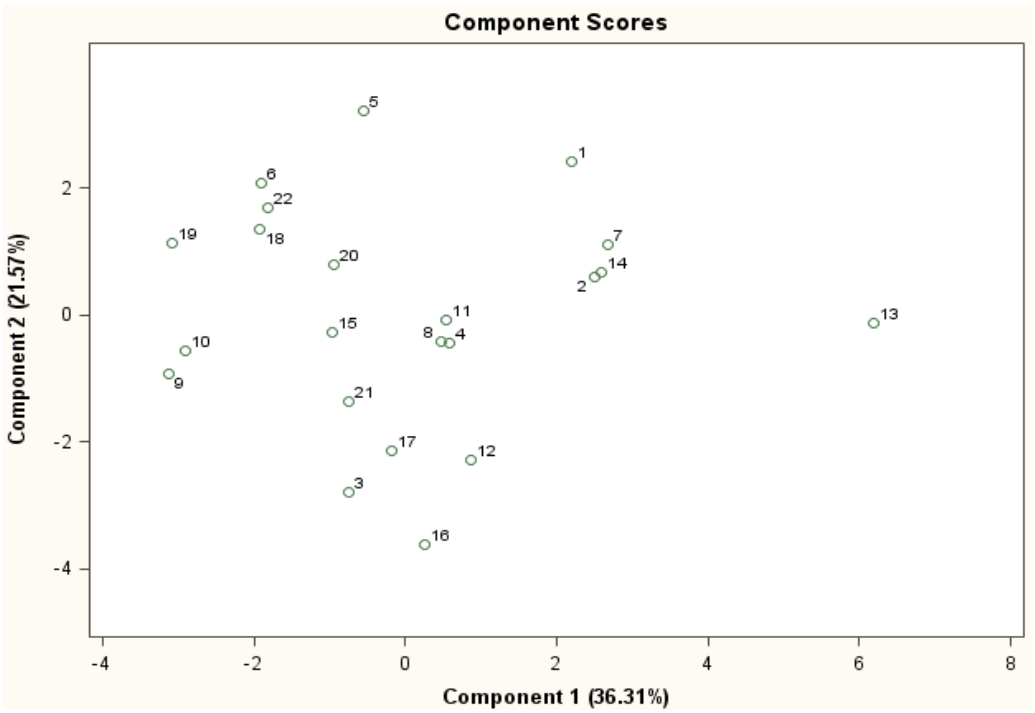

Figure 3. Configuration of the 22 strawberry genotypes under principal component axes 1 and 2.

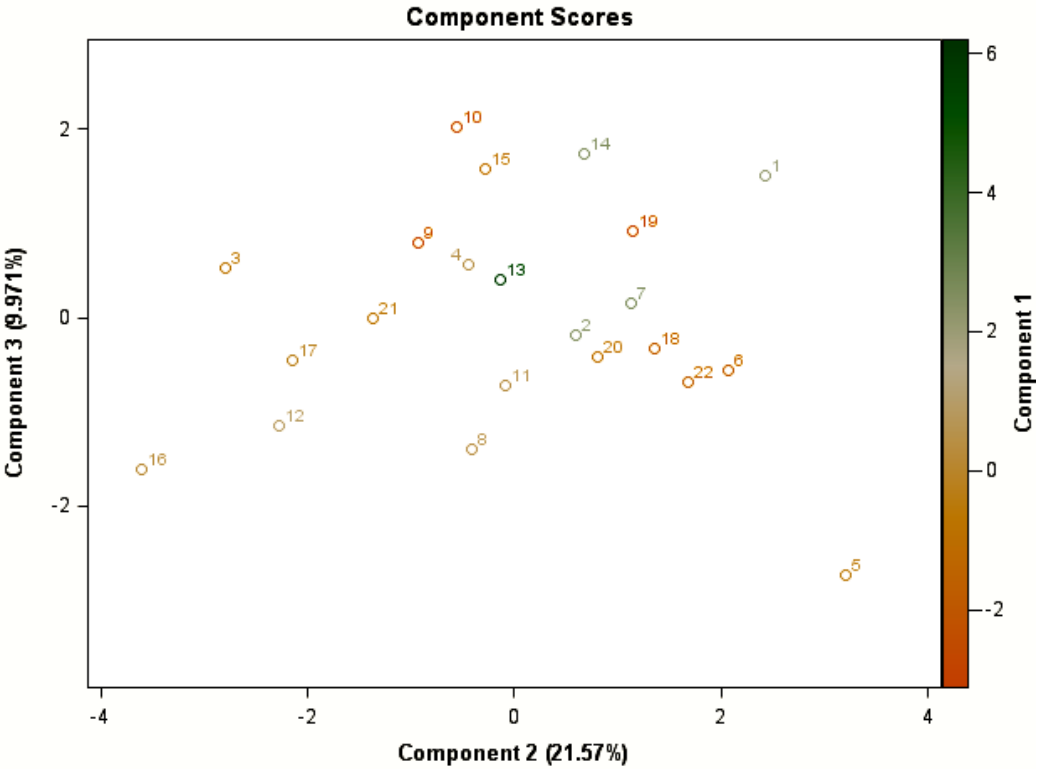

Figure 4. Configuration of the 22 strawberry genotypes under principal component axes 1, 2 and 3.

and use in future breeding programs.

Nevertheless, morphological descriptors, which are environmentally influenced, are not enough to identify strawberry genotypes because the differences among them are often ambiguous. Biochemical (Al-Said et al., 2009), as well as molecular (Jbir et al., 2008) markers are required to complete this study in order to evaluate and better estimate diversity among *Fragaria* × *ananassa* genetic resources.

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