academicJournals

Vol. 8(45), pp. 5599-5606, 21 November, 2013
DOI:10.5897/AJAR2013.7969
ISSN 1991-637X ©2013 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Determination of genetic diversity in onion (*Allium cepa* L.) by multivariate analysis under long day conditions

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Accepted 12 November, 2013

The present investigation was conducted on thirty eight accessions of onion at the experimental farm of Central Institute of temperate Horticulture to study the variability and their interrelationship and divergence pattern based on quantitative and qualitative traits during 2009 to 2011. Multivariate analysis was used to classify 38 long day onion accessions. All accessions were grouped into six different clusters. The highest inter cluster distance was observed between IV and V and lowest between II and VI. Based on cluster means the important cluster was I for plant height and percent marketable bulbs cluster IV for mean yield, total yield and equatorial diameter and cluster III for the number of leaves, collar thickness and average bulb weight. Accordingly, parents could be selected for hybridization. Principal component analysis, first two principal component accessions accounted for 59.29% of the variance among 38 accessions. The greater part of the variance was accounted for other traits such as plant height, collar thickness, percent marketable bulb yield, polar diameter of the bulb and neck thickness. The high diversity found in the accessions showed its great potential for improving qualitative as well as quantitative traits in long day onion.

Key words: Genetic divergence, multivariate analysis, hybridization, principal component analysis.

INTRODUCTION

Onion (*Allium cepa* L.) is one of the important spice and vegetable crop grown in temperate (Brewster, 1990), sub-tropical (Corgan and Kedar, 1990) and tropical climate (Currah and Proctor, 1990) throughout the world. It is cultivated year round but maximum during *Rabi* season in our country. The crop is grown for variety of purposes from kitchen to factory made products/food and also for dehydration. It is valued for its distinct pungent flavor and its essential ingredients cuisine. It is consumed round the year by all the sections of people through-out the world due to healing properties of onion in case of cardiac diseases, rheumatism, cancer, digestive disorders, blood sugar and prolong cough. It is a photo-sensitive crop and on the basis varieties are divided into short day and long day types. Long day types are high yielder but have poor shelf life whereas short day types have better shelf life with the low yielding capacity. So, development of high yielding varieties with good quality traits is needed now days. To improve the yield through selection, information on the nature and magnitude of variability present in a population is an important pre-requisite for starting any breeding programme. For a successful breeding program, the presence of genetic diversity and variability play a vital role. Genetic diversity is essential to meet the diversified goals of plant breeding such as breeding for increasing yield, wider adaptation, desirable quality, pest and disease resistance. Selection of genetically diverse parents in any breeding programme is of immense importance for successful recombination breeding (Arunachalam, 1981). The genetic divergence analysis estimates the extent of diversity existed among selected genotypes (Mondal, 2003). Precise information

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Table 1. Long day onion accessions with code used in the study.

S/N	Germplasm	Code	S/N	Germplasm	Code	S/N	Germplasm	Code
1	ARBO-1001	1	14	BRBO-1006	14	27	BRBO-1019	27
2	ARBO-1002	2	15	BRBO-1007	15	28	BRBO-1021	28
3	ARBO-1003	3	16	BRB0-1008	16	29	BRBO-1022	29
4	ARBO-1004	4	17	BRBO-1009	17	30	BRBO-1024	30
5	ARBO-1005	5	18	BRBO-1010	18	31	BRBO-1025	31
6	ARBO-1006	6	19	BRBO-1011	19	32	BRBO-1026	32
7	ARBO-1007	7	20	BRBO-1012	20	33	BRBO-1029	33
8	ARB0-1030	8	21	BRBO-1013	21	34	BRBO-1030	34
9	BRBO-1001	9	22	BRBO-1014	22	35	BRBO-1023	35
10	BRBO-1002	10	23	BRBO-1015	23	36	BRBO-1028	36
11	BRBO-1003	11	24	BRBO-1016	24	27	BRBO-1031	37
12	BRBO-1004	12	25	BRBO-1017	25	38	BRBO-1027	38
13	BRBO-1005	13	26	BRBO-1018	26			

on the nature and degree of genetic diversity helps the 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. The parent identified on the basis of the divergence analysis would be more promising. Mohanty (2001a); Mohanty and Prusty (2002) have reported some results in onion. The D^2 statistic provides a quantitative measure of genetic divergence among populations and assists in classifying genetic stocks into district groups which is further helpful for evolving superior genotypes. When 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. The principal component analysis (PCA), one of multivariate analysis methods, showed which of the traits were decisive in genotype differentiation (Kovacic, 1994). PCA enables easier understanding of impacts and connections among different traits by finding and explaining them. The present investigation is an attempt to assess the magnitude of genetic diversity in bulb yield potential of onion accessions and to isolate the diverse ones according to their genetic affinity for future improvement programme.

MATERIALS AND METHODS

The present research study was carried out at the research farm of Central Institute of Temperate Horticulture (CITH), Srinagar, for two years that is, 2009-2010 to 2010-2011. Thirty eight accessions (Table 1) were taken for the study. Seedlings of 50 days old were transplanted in main field during winter Rabi. Each accession was grown in 10 row of two meter length with a spacing of 10 × 15 cm. The experiment was conducted under randomized block design, replicated three times and pooled data of two years were analyzed as per the method suggested by Gomez and Gomez (1984). The observations were recorded on randomly selected plants from each plot for 11 characters namely plant height (cm), no. of leaves, collar thickness (mm), mean yield (t/ha), % marketable bulbs, total yield (q/ha), polar diameter (mm), equatorial diameter (mm), neck thickness (mm), average bulb weight (g), T.S.S (%). Plot means over the replications were used for the statistical analysis. The total soluble solids (TSS) were determined with digital refractometer calibrated using distilled water. Results were reported as ◦Brix at 22°C (Ruck, 1963). Genetic diversity was studied using Mahalanobis (1936) generalized distance (D^2) extended by Rao (1952). Clustering of genotypes was done according to Tocher's Method Rao (1952). Average intra-cluster distance was calculated by the following formula as suggested by Singh and Choudhary (1985). Trait variability analysis was performed by the principal component analysis (PCA) method, with the number of principal components being chosen based on the screen test (Kovacic, 1994). Agglomerative Hierarchical cluster analysis was used to determine differences and similarities among the genotypes, and the distance measure used was Euclidean distance as the parameter that best reflects the differences existing among the genotypes (Kendall, 1980). All statistical analysis was carried out based on eleven agro-morphological quality traits using XL STAT-2011 and SAS 9.2 software SAS Institute (2011) (Table 1).

RESULTS AND DISCUSSION

The data on variability parameter are presented in Table 2. The lowest values of standard deviation were recorded in the case of the equatorial diameter of the bulb (0.492) followed by the polar diameter of the bulb (0.616). The highest standard deviation value was that for the total yield (80.19). The coefficients of variation were the lowest for the percent marketable yield (2.14) followed by plant height (8.03) however, highest coefficient of variation value was for neck thickness (32.60) followed by total yield (22.49) and average bulb weight (21.48). Based on D^2 value estimates of genetic divergence the 38 onion accessions were grouped into six distinct clusters (Figure

Table 2. Variability of physico-chemical traits in thirty eight accessions of onion.

Character	Range		Mean	Std. deviation	CV%
	Minimum	Maximum			
Plant Height (cm)	41.210	56.220	48.523	3.897	8.031003
No. of leaves	7.000	12.110	9.227	1.434	15.54207
Collar thickness (mm)	11.130	17.110	14.461	1.501	10.37771
Mean yield (t/ha)	223.630	477.000	344.971	70.884	20.54777
% Marketable bulbs	91.170	98.330	94.832	2.031	2.142031
Total yield (q/ha)	136.220	491.000	356.467	80.199	22.49837
Polar dia. (mm)	4.170	6.940	6.056	0.616	10.16765
Equatorial diameter	4.200	6.020	5.267	0.492	9.337278
Neck thickness (mm)	1.210	17.890	13.811	4.503	32.60526
Av. Bulb wt. (g)	58.620	155.060	103.570	22.255	21.48756
T.S.S (%)	8.770	14.620	10.761	1.423	13.21995

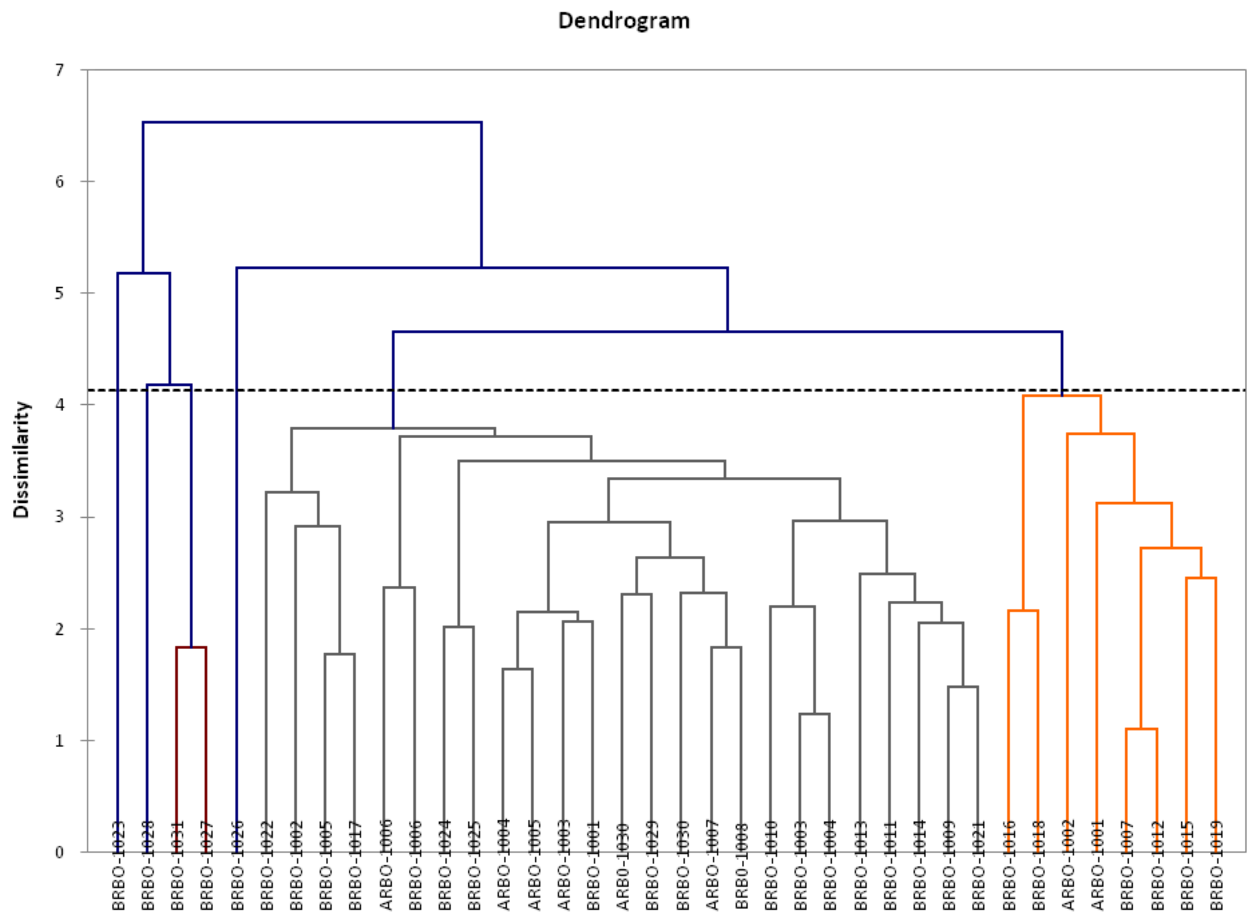

Figure 1. Dendogram for the 38 accessions produced by average distance clusters analysis; clusters are based on morphological traits (scale: Euclidean distance).

1 and Table 4). A wide range of diversity was observed in the experimental material for the majority of the characters studied including physico-chemical traits. Cluster II consisted of a maximum of 25 genotypes (65.78%), Cluster I consisted 8 genotypes (21.05%), cluster VI consisted 5 genotypes (5.216%) and Cluster III,

Table 3. Average intra- (bold face) and inter-cluster distance (D^2) of 38 onion genotypes.

S/N	1	2	3	4	5	6
1	**47.61**	160.021	95.827	144.621	302.766	115.506
2		**64.24**	214.728	297.364	145.077	53.234
3			**0.00**	141.133	357.159	178.343
4				**0.00**	435.974	255.424
5					**0.00**	191.101
6						**44.39**

IV and V consisted 1,1,1, genotypes 2.63, 2.63 and 2.63% respectively. Cluster II had the highest intra cluster distance (64.24) indicating the high divergence among the genotypes of the cluster while considering the inter cluster distance minimum distance (53.23) was noticed between II and VI (Table 4). Maximum inter cluster distance (435.97) was noticed between IV and V followed by distance 357.15 between (III and V) and distance 297.36 between II and IV (Table 3). According to Ghaderi et al. (1984), increasing parental distance implies a great number of contrasting alleles at the desired loci, and to the extent that these loci recombine in the F2 and F3 generation following a cross of distantly related parents, the greater will be the opportunities for the effective selection for yield factors. Thus, crossing of genotypes from these clusters with other clusters may produce higher amount of heterotic expression in the first filial generations (F1's) and wide range of variability in subsequent segregating (F2) populations. The distribution of genotypes into different clusters showed no uniformity with respect to their origin. Thus, ruling out the association between geographical distribution and genetic divergence. Similar results have been reported by Lee et al. (1996), Mohanty (2001b) and Khar et al. *(*2006) in onion. Based on cluster means the important cluster was I for plant height and percent marketable bulbs. Cluster IV for mean yield, total yield and equatorial diameter and Cluster III for number of leaves, collar thickness and average bulb weight. The maximum distances existed between Cluster IV and V, III and V and II and IV. From the results it concluded that genotypes I for plant height and percent are marketable bulbs. Genotypes for mean yield, total yield and equatorial diameter from Cluster IV and for number of leaves, collar thickness and average bulb weight from Cluster III could be selected as parents for hybridization programme. Crosses involving parents belonging to more divergent clusters would be expected to manifest maximum heterosis and wide variability in genetic architecture (Singh et al., 1987).

In the present study, Cluster II was more divergent than the others. However, the chance of getting segregates with high yield level is quite limited when one of the characters has a very low yield level. The selection of parents should also consider the special advantage of each cluster and each genotype within a cluster depending on specific objective of hybridization (Chahal and Gosal, 2002). Thus, crosses involving Cluster IV and V with any other cluster except Cluster II and VI are suggested to exhibit high heterosis and could result in segregates with higher onion bulb yield. Principal component analysis (PCA) reflects the importance of the largest contributor to the total variation at each axis of differentiation (Sharma, 1998). The eigen values are often used to determine how many factors to retain. The sum of the eigen values is usually equal to the number of variables. Therefore, in this analysis the first factor retains the information contained in (3.83) of the original variables. The coefficients defining the eleven principal components of these data are given in Table 5. The coefficients are scaled, so that they present correlations between observed variables and derived components. Three principal components, PC1 to PC3, which are extracted from the original data and having latent roots greater than one, accounting nearly 71.03% of the total variation. Suggesting these principal component scores might be used to summarize the original 11 variables in any further analysis of the data. Out of the total principal components retained, PC1, PC2, and PC 3 with values of 34.82, 24.44 and 11.74% respectively contributed more to the total variation. According to Chahal and Gosal (2002) characters with largest absolute value closer to unity within the first principal component influence the clustering more than those with lower absolute value closer to zero. Therefore, in the present study, differentiation of the accessions into different clusters was because of relatively high contribution of few characters rather than small contribution from each character. Accordingly, the first principal component had high positive component loading from plant height, collar thickness, percent marketable bulb yield, polar diameter of bulb and neck thickness of bulb and high negative loading from TSS while remaining traits in this PC1 did not contributed rather their effects were distributed among other PCs. The positive and negative loading shows the presence of positive and negative correlation trends between the components and the variables. Therefore, the above mentioned characters which load high positively or negatively contributed more to the diversity and they were the ones that most differentiated

Table 4. Cluster means for eleven characters in genotypes.

Characters	Cluster					
	1,2,15,20,23,24,26,27(21.05)	3,4,5,6,7,8,9,10,11,12,13,14,16,17,18,19,21,22,25,28,29,30,31,33,34(65.78)	32(2.63)	35(2.63)	36(2.63)	37,38(5.25)
P.Ht (cm)	50.521	48.785	49.780	42.440	45.310	41.270
No. of leaves	10.030	9.189	11.670	7.000	7.000	7.500
Collar thickness (mm)	15.131	14.592	16.620	11.940	12.000	11.545
Mean yield (t/ha)	271.386	373.444	228.700	230.000	477.000	333.000
% Marketable bulbs	95.173	94.960	95.080	92.670	93.000	93.750
Total yield (q/ha)	272.623	390.592	237.920	136.220	491.000	367.410
Polar dia. (mm)	5.746	6.338	5.500	5.320	6.000	4.445
Equatorial diameter	4.643	5.455	5.440	4.700	5.710	5.400
Neck thickness (mm)	14.596	15.498	14.840	1.900	1.210	1.310
Av. Bulb wt. (g)	76.689	112.304	155.060	58.620	115.000	92.945
T.S.S (%)	10.374	10.349	13.240	14.620	12.000	13.665

Table 5. Latent vectors for eight traits of 11 onion genotypes.

Characters	Eigen vectors		
	PRIN1	PRIN2	PRIN3
P.Ht (cm)	0.282665	-0.319700	-0.099105
No. of leaves	0.215042	-0.381590	0.380009
Collar thickness (mm)	0.320344	-0.340811	0.124338
Mean yield (t/ha)	0.271941	0.436897	-0.126374
% Marketable bulbs	0.216863	-0.118513	0.159304
Total yield (q/ha)	0.272661	0.444949	-0.210002
Polar dia. (mm)	0.416135	0.079129	-0.056084
Equatorial diameter	0.233816	0.275704	0.541252
Neck thickness (mm)	0.382032	-0.239509	-0.138899
Av. Bulb wt. (g)	0.311144	0.291892	0.318820
T.S.S (%)	-.324521	0.091024	0.572558
Eigen values	3.830	2.692	0.3
T. varience	34.82	24.48	11.17
Cumulative percentage varience	38.42	59.29	71.03

the clusters The genotypes in the PC1 were more likely to be associated with plant height, collar thickness, percent marketable bulb yield, polar diameter of bulb and neck thickness of bulb whereas the genotypes with mean bulb yield/ha and total yield were contributing second PC

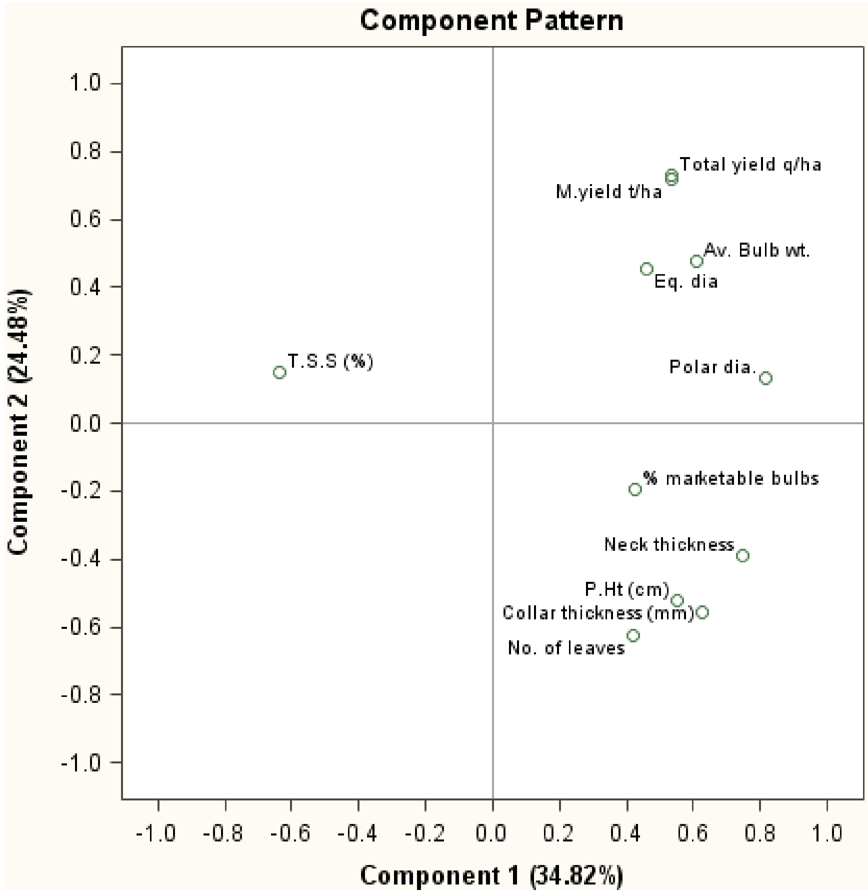

Figure 2. Relationships among 11 morphological and yield traits in 38 onion accessions shown by a 2D scatter for first two principal components.

(Figure 2). The traits which contributed more positively to PC3 were numbers of leaves, equatorial diameter average bulb weight and TSS (Figure 3). In the component pattern diagram (Figures 2 and 3) correlation between variables and the principal component depicted and it has been found the total yield, mean yield, average bulb weight and equatorial diameter are positively correlated, polar diameter and T.S.S are non significantly correlated however percent marketable bulbs, neck thickness, plant height, collar thickness, number of leaves are strongly negatively correlated. The configuration of the thirty eight onion genotypes along the first three principal component axis are shown in Figure 1. The coordination of the genotypes on all the axis together (Figure 4) revealed that genotypes 2, 27, 32, 35, 36, 37 and 38 found to be most distinct genotypes for the characters studied. Usually it is customary to choose one variable from these identified groups. Hence, for the first group polar diameter of bulb is best choice, which had the largest loading from component ones, total yield for the second, and TSS for the third group. The characters contributed positively to first three principal components could be given due consideration while selecting the best genotypes without losing yield potential.

The present investigation provided considerable information useful in genetic improvement of onion. Genotypes grouped into Cluster IV and V showed maximum inter cluster diversity. From cluster mean values, genotypes in Cluster I, III and IV deserve consideration for their direct use as parents in hybridization programs to develop high yielding long day onion varieties. There is significant genetic variability among tested genotypes that indicates the presence of excellent opportunity to bring about improvement through wide hybridization by crossing genotypes in different clusters.

Further studies on the influence of environment and agronomic practices on the genetic potential of the varieties in different long day onion environment are necessary. This is helpful to stratify the environments based on quality and yield stability. Generally, the development of long day onion varieties possessing equatorial diameter, polar diameter, average bulb weight, better T.S.S and yield.

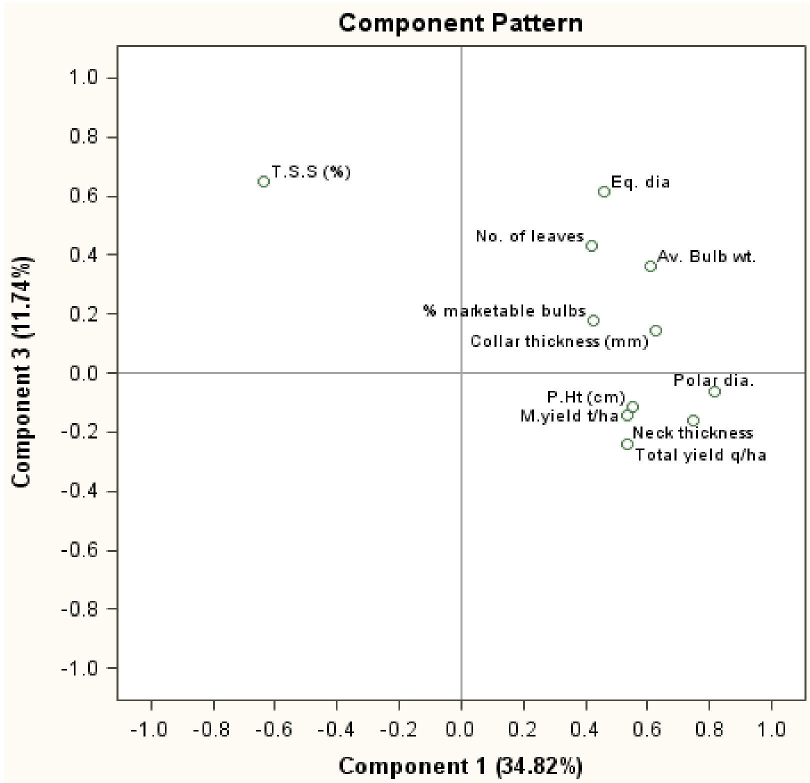

Figure 3. Relationships among 11 morphological and yield traits in 38 onion accessions shown by a 2D scatter for first and third principal components.

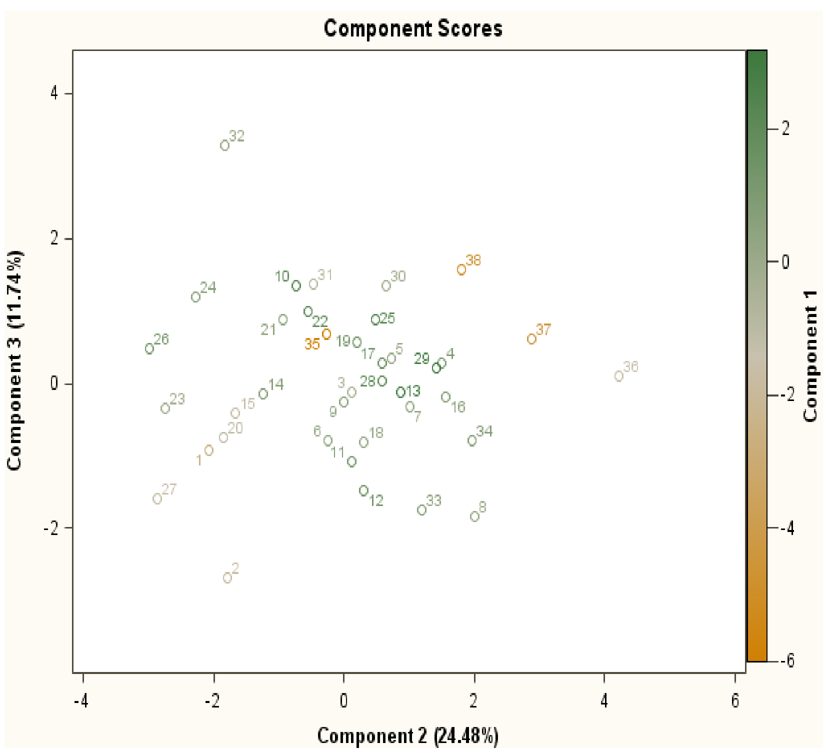

Figure 4. Relationships among 38 onion accessions shown by a 2D scatter for first three principal components based on morphological and yield traits (code for respective accessions given in Table 1).

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