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GGE Biplot Analysis for Characterization of Garlic (*Allium sativum* L.) Germplasm Based on Agro-Morphological Traits

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ABSTRACT

A breeding strategy in any crop improvement depends upon line selection, either for future varieties or for useful parents for hybridization, must be based on multiple breeding objectives (or traits). It is hypothesized that varieties cannot have any major defects, while parents must have outstanding levels in at least one trait. The objective of this paper is to propose a comprehensive multi-trait selection procedure that coherently combines independent selection. The present study was carried out to characterize a germplasm collection of 25 garlic (*Allium sativum* L.) genotypes representing the Indian collection from different garlic-growing regions, maintained at Punjab Agricultural University, Ludhiana. GGE biplot analyses were used for multi-trait selection to identify accessions on the basis of collective desirable trait combinations in an individual genotype. Seven genotypes (AC-50, AC-200, JG-03-263, PG-17, PG-1, PG-18 and RAUG-5) having combinations of desirable morphological variation were identified. A trait association study indicated three traits *viz.* plant height, bulb length and number of cloves per bulb, to be the main determinants of bulb weight. Bulb length and clove length exhibited a maximum positive correlation. Ranking on the basis of overall performance and trait stability analysis of genotypes for the studied traits led to the identification of one genotype PG-18, having a balanced performance for all traits and high yield potential. In addition, PG-1, PG-19, PG-17, NRCWG-2, PG-32, JGL-96-198, PG-30 and NRCRG-1 were also identified as desirable genotypes for the studied characters. This study indicates an extensive range of variability for various desirable traits and would help breeders to select various characters responsible for most of the variation to develop better varieties. Those cultivars possessing desirable traits can be further exploited for securing high yield as well as in targeted breeding.

Keywords: genotype, multi-trait selection, morphological variation, selection, trait association

INTRODUCTION

Garlic (*Allium sativum* L. 2n = 16) belongs to the family Alliaceae, is the second most widely used *Allium* next to onion (Rubatzky and Yamaguchi 1997). The centre of origin of garlic was unclear for a long time although garlic has been widely cultivated since ancient civilization. It is originated on the north-western side of the Tien-Shan Mountains of Kirgizia in the arid and semi-arid areas of central Asia (Kazakhstan) (Etoh and Simon 2002). The Food and Agriculture Organization (FAO 2010) reported that in India, garlic was grown on an area of 164860 ha with a production of about 833970 tonnes and productivity was 50587 Hg/ha.

Garlic is commonly used for culinary purposes with well known medicinal value. It is a rich source of carbohydrates, proteins and phosphorus and ascorbic acid content was reported to be very high in green garlic. The thiosulfinates are responsible for the flavour and pungency of fresh garlic (Natale *et al*. 2005). In garlic bulbs, alliin is the predominant flavour precursor, while allicin is consequently the major thiosulfinates accounting for 60-95% of total thiosulfinates (Block *et al*. 1992). Garlic is a vegetatively propagated crop, usually propagated through cloves. Despite sexually sterile in nature, it displays an extensive morphological variation (Bradley *et al*. 1996) and it has been presumed that the vast diversity observed in cultivated garlic may be due to variation generated from sexual reproduction in the wild crop (Simon 2004). Likewise, several researchers (Zahedi 2007; Stavelikova 2008; Gvozdanović-Varga *et al*. 2009; Teweldebrha 2009; Volk and Stern 2009) reported the existence of wide phenotypic and genotypic diversity in morphological traits, bulb yield and yield components and quality in garlic.

Morphological characterization is a useful indicator of evaluating the agronomic worth of a genotype of any crop species. Effective improvement programme in vegetatively propagated crop depends upon the availability of sufficient genetic variability, so that selection could be operated. Further, the knowledge of the extent and nature of genetic correlation among different characters provides breeder the valuable information regarding the correlated response to selection and also helps in formulating efficient scheme of multiple trait selection, as it provides means of direct and indirect selection of component characters. Garlic grown in India possess wide diversity and includes, bolters and non-bolters, various bulb shapes, sizes and colours. Therefore, the present study was undertaken with the objective to characterize garlic germplasm maintained at Punjab Agricultural University, Ludhiana, India, on the basis of different morphometric traits using GGE biplot methodology for its effective utilization in breeding programmes for productivity enhancement.

MATERIALS AND METHODS

Plant material and research area

The experimental material comprised of 25 genotypes (**Table 1**) of garlic representing diverse geographic regions of 9 states of India. Of these 25 genotypes; ten genotypes (PG-30, PG-1, PG-19, PG-18, PG-31, PG-26, PG12, PG-3, PG-17, PG-32) from Punjab, five

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Table 1 Basic statistics and genetic parameters of 12 morpho-physiological characters of 25 garlic genotypes.

Character	GM	Range	CV (%)	PCV (%)	GCV (%)	Heritability (%)	GA	GA (% mean)
Plant height (cm)	64.28	49.62-75.77	5.14	10.25	8.86	74.81	10.15	15.79
Number of leaves per plant	8.31	7.30-9.45	3.27	8.68	8.04	85.86	1.28	15.35
Bulb weight (g)	23.58	15.00-33.63	11.25	20.39	17.01	69.59	6.89	29.24
Bulb length (cm)	3.47	2.89-4.19	7.25	11.44	8.85	59.83	0.49	14.10
Bulb diameter (cm)	3.81	2.98-4.27	4.76	10.85	9.74	80.71	0.69	18.03
Number of cloves per bulb	22.82	7.41-32.67	5.98	24.74	24.01	94.16	10.95	47.99
Clove weight (g)	1.13	0.66-2.33	15.80	35.30	31.57	79.97	0.66	58.15
Clove length (cm)	3.13	2.50-4.17	6.35	12.92	11.25	75.86	0.63	20.19
Clove diameter (cm)	0.91	0.66-1.29	18.76	25.39	17.11	45.41	0.22	23.75
Bulbils	-	P*/A*	-	-	-	-	-	-
Position of bulbils	-	E, SE, B	-	-	-	-	-	-
Number of bulbils per plant	3.32	0-7.19	9.86	79.19	78.58	98.45	5.33	160.61

GM General mean, CV coefficient of variation, PCV and GCV phenotypic and genotypic coefficients of variation, heritability (broad sense) and GA genetic advance, GA (% mean) genetic advance as percent of means

*/**- Bulbils (Present/Absent) E - Emergent, SE - Semi-emergent, B - Both (Emergent and Semi-emergent)

Table 2 Analysis of variance for different characters studied in garlic (*Allium sativum* L.).

Source	d.f	Plant height	No of leaves per plant	Bulb weight	Bulb length	Bulb diameter	Number of cloves per bulb	Clove weight	Clove length	Clove diameter	Number of bulbils per plant
Genotypes	24	108.31*	141**	55.32**	0.35*	0.45**	91.96**	0.41*	0.41*	0.10*	20.51**
Error	46	10..96	0.07	7.03	0.06	0.03	1.86	0.03	0.04	0.03	0.11

**,* significant at 1% and 5% level of significance

(AC-200, NRCRG-1, AC-50,NRCWG-2, G-189) from Maharashtra, four (JNDG-213, JNDG-219, JGL-96-198, JG-03-263) from Gujarat and single genotypes (KGS-2-PEW, G-282, G-50, DARL-52, RAUG-5, G-1) from five states namely Uttar Pradesh, Tamil Naidu, Haryana, Uttaranchal, Bihar and Delhi. The material maintained at Vegetable Experimental Farm (Latitude 30° 54' N, longitude 75° 27' E), Department of Vegetable Crops, Punjab Agricultural University, Ludhiana. The soil of the research farm sandy loam to clayey with normal reaction (pH 7.8 to 8.5).

Experimental design

The genotypes were planted in *rabi* crop season (15 September, 2008) with three replications during 2008-2009. Each test genotype was assigned randomly to each replication in a randomized block design (RBD). Each genotype was planted in a plot size 3 × 3 m at a recommended 15-cm row-to-row distance and 7.5 cm plant-to-plant distance. Standard agronomic practices were followed to raise the healthy crop. Five competitive plants of each genotype per replication were randomly tagged and used for recording data of the ten morpho-agronomical traits *viz.* plant height, number of leaves per plant, bulb weight, bulb length, bulb diameter, number of cloves per bulb, clove weight, clove length, clove diameter and number of bulbils per plant.

Statistical analysis

Recorded observations were subjected to analysis of variance (ANOVA) through CPCS 1 software (Punjab Agricultural University). The ANOVA indicated a significant genotypic variation, which was taken into further consideration using the GGE biplot software and SPAR 2.0 (Indian Institute of Statistical Research) with objective of characterizing the available germplasm to identify genotypes with desirable traits. The biplot graphs were made using GGE biplot software (Yan 2001), which is based on principal component analysis (PCA). GGE biplot analysis was used to evaluate all 25 genotypes for 10 quantitative traits to visual analysis of the genotype by trait data. GGE biplot is a Windows application that performs biplot analysis of two-way data that assume an entry × tester structure. GGE biplot analyzes the data and outputs the results as an image. It allows interactive visualization of the biplot from various perspectives (Yan 2001).

To display the genotype by trait two-way data in a biplot, the following formula is used,

$$\frac{Tij - Tj}{Sj} = \lambda 1\ \zeta i1\ Tj1 + \lambda 12 \zeta i2\ Tj2 + \varepsilon ij \quad (1)$$

where *Tij* is the average value of genotype *i* for trait *j*, *Tj* is the average value of trait j over all genotypes, Sj is the standard deviation of trait *j* among the genotype averages; ζi1 and ζi2 are the PC1 and PC2 scores, respectively, for genotype *i*; Tj1 and Tj2 are the PC1 and PC2 scores, respectively, for trait *j*; and ε*ij* is the residual of the model associated with the genotype i in trait j. Equation (1) is a principal component analysis of standardized data with two principal components. Because different traits use different units, the standardization is necessary to remove the units. A genotype × trait (GT) biplot was constructed by plotting the PC1 scores against the PC2 scores for each genotype and each trait. All biplots of all six groups presented in this study were generated by using "GGE biplot" software (Yan 2001).

RESULTS AND DISCUSSION

The selection of parents based on phenotypic trait performance is an essential step for the success of any crop breeding programme. Among the different screening techniques available, the characterization, cataloging and estimation of genetic variation for various desirable traits is important and routine practices of an established breeding programme. Estimates of different basic statistical and genetic parameters like mean, range, genotypic and phenotypic coefficient of variability, heritability and genetic advance as well as percentage of mean for all ten characters under study are presented in **Table 1**. The analysis of variance for the characters under study revealed that the mean square estimates due to genotypes were significant for all the characters studied (**Table 2**). Genotypic coefficient of variation (GCV) would be more useful for the judgment of the variability than the phenotypic variability, since it depends upon the heritable fraction of the total variability. The estimated genotypic coefficient of variability for all the characters under study ranged from 8.04-78.58. Maximum expressions of genotypic coefficient of variation was observed in case of number of bulbils per plant (78.58) followed by clove weight (31.57), number of cloves per bulb (24.01), clove diameter (17.11) and bulb weight (17.01). Therefore, these traits had high heritable proportion and selection should be practiced for exploitation of that particular trait. Moderate expression of genotypic coefficient of variation was observed for clove length (11.25). Minimum expressions of phenotypic coefficients of variation were observed for bulb diameter (9.74), plant height (8.86), bulb length (8.85) and number of leaves per plant (8.04).

The GCV and PCV estimates are comparable suggesting that the environmental influence on most of the traits was low indicating the greater role of genetic factors causing variability in these characters. These observations indicated ample scope for improvement of the above traits by undergoing selection from their phenotypic values. Heritability expressed as percentage ranged from 45.41 to 98.45. High heritability estimates were obtained for number of bulbils per plant (98.45), number of cloves per bulb (94.16) and number of leaves per plant (85.86). This high value of heritability also suggests that the selection for high yield must be performed on these yield contributed traits. The moderate heritability estimates were obtained for bulb weight (69.59), bulb length (59.83) and clove diameter (45.41). The highest genetic advance as percentage of mean (genetic gain) was predicted high for number of bulbils per plant (160.61) followed by clove weight (58.15) and number of cloves per bulb (47.99). Moderate genetic advance estimates were obtained for bulb weight (29.24), clove diameter (23.75), clove length (20.19) and bulb diameter (18.03). Low genetic advance was observed for plant height (15.79), number of leaves per plant (15.35) and bulb length (14.10). It was evident that the bulbils per plant had highest heritability, GCV and genetic advance suggesting that any improvement in this character will lead to an increase in the yield per plant.

Genotypic evaluation based on multiple traits

A genotype by trait biplot is a useful tool for exploring multiple trait data and can aid in multitrait selection because it graphically displays the trait associations across, and the trait profiles of, the genotypes. Germplasm set was characterized on the basis of 10 economically important agro-morphological traits through GGE biplot analysis. In this analysis, data were not transformed ("Transform = 0"), scaled ("Scaling = 1") and were tester/ trait centered ("Centering = 2"). The biplot graph generated for "relationship between the characters" was based on character-focused singular value partitioning ("SVP = 2") and therefore was thought appropriate for visualizing the relationships among traits. The principle components makeup biplot through PC I and PC II together explained 59.5% (PC I = 32.9%, PC II = 26.6%) of the total variation. In present analysis number of cloves per bulb, plant height, clove weight, bulb length, clove length, clove diameter, bulb diameter, bulb weight were the most discriminative traits, whereas, number of leaves per plant and number of bulbils per plant were least informative for characterization of this set of germplasm.

Identification of desirable genotypes for yield and yield-contributing traits

To identify and characterize potential genotypes carrying the trait of interest or package of desirable traits 'which won where' biplot were constructed (**Fig. 1**) (Yan and Tinker 2007). High or low performing vertex accessions along with their trait specificity were identified by genotype by trait biplot. Some of these had maximum contribution for yield component traits *viz.* high bulb and clove weight, bulb diameter, clove and bulb length, etc. In the polygon, five genotypes namely PG-18, PG-17, PG-1, JG-03-263, AC-200, AC-50 and RAUG-5 were identified with high or low values for evaluated traits therefore designated vertex genotypes. A perpendicular line that passes through the origin of the biplot and intersects divides the biplot into different sectors, each sector having its own best/poor performing genotypes. Genotype PG-18 was identified as desirable for diameter and weight of bulb, clove length and bulb length while AC 200, AC 50 and PG 31had low value for these traits. PG-17 had high value for BWT, BD, PHT and NC. Genotype RAUG-5 was desirable for CWT and CD whereas JG-03-263, PG-17, PG-1, AC-200, PG-26, G-189 accounted low value for these traits. A clove per bulb was highest in JG-03-263 while RAUG-5 was accounted for lowest. PG-1 was identified to be desirable for number of had high BPP but low in AC-200, AC-50 and RAUG-5.

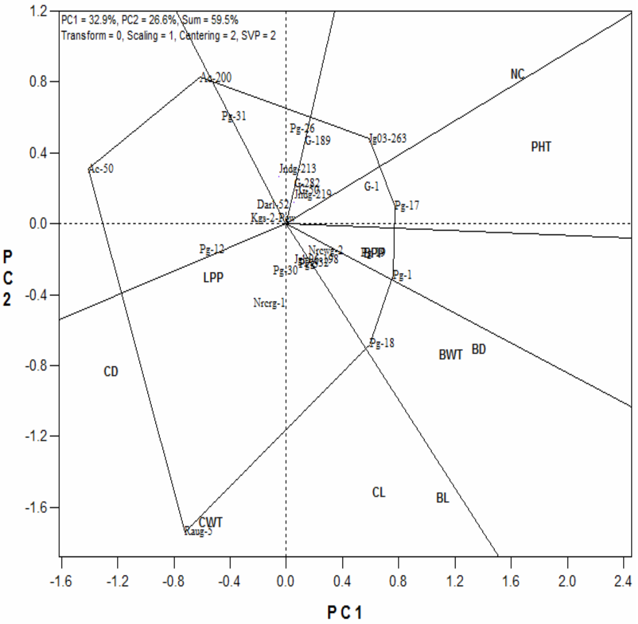

Fig. 1 Polygon view of the genotypes and characters biplot of 25 garlic genotypes.

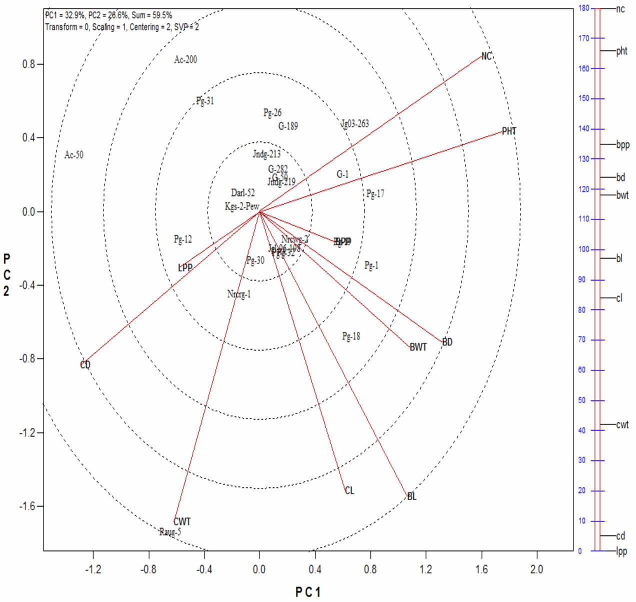

Fig. 2 Relationship/correlation among different characters.

Association among traits

Association/correlation information among different characters is required to obtain the expected response of these characters when selection is applied to the character of interest in a breeding program. The trait focused biplot is shown in **Fig. 2** was used to investigate association among the traits and to identify the potential related traits for indirect selection. The lines that connect the biplot origin and characters are called character vectors and the cosines of the angles between the vectors of the two traits approximate the extent of correlation between them (Kroonenberg 1995; Yan *et al.* 2000). The interrelationship of the investigated characters is presented graphically along with linear map of characters to provide the information about the relationship among the characters (**Fig. 2; Table 3**). The maximum positive correlation (r=**0.795) was found between bulb length and clove length whereas bulb diameter and clove weight had lowest positive correlation

Table 3 Relationship/correlation among different characters.

Row/Col	BWT	PHT	LPP	BL	BD	NC	CWT	CL	CD	BPP
BWT	1	0.389*	0.151	0.565**	0.37	0.384*	0.131	0.256	-0.093	-0.008
PHT		1	-0.272	0.32	0.44	0.791**	-0.428	0.093	-0.642	0.155
LPP			1	-0.088	-0.322	-0.123	0.146	0.077	0.207	-0.011
BL				1	0.594**	0.075	0.463*	0.795**	-0.044	0.101
BD					1	0.356	0.061	0.367	-0.115	0.21
NC						1	-0.631	-0.09	-0.603	0.232
CWT							1	0.469	0.51	0.135
CL								1	-0.049	0.108
CD									1	-0.198
BPP										1

*r for $P < 0.05$ is 0.375, **r for $P < 0.01$ is 0.490

BWT- Bulb weight (g), PHT- Plant height (cm), LPP- Number of leaves per plant, BL- Bulb length (cm), BD- Bulb diameter (cm), NC- Number of cloves per bulb, CWT- Clove weight (g), CL- Clove length (cm), CD- Clove diameter (cm), BPP- Number of bulbils per plant

(r=0.061). In support to our study bulb yield per plant showed positive and significant phenotypic correlation with weight of clove and cloves per bulb (Tsega *et al.* 2010). Godhani and Singh (2000), Naruka and Dhaka (2004), and Dubey *et al.* (2010) also reported similar significant positive correlation between bulb yield with bulb weight and bulb size. In the present analysis, bulb weight was significantly and positively correlated with three traits viz. plant height (0.389), bulb length (0.565) and number of cloves per bulb (0.384) indicating that any improvement in any one of these characters will lead to an increase in the bulb weight and ultimately yield. The correlation studies revealed that, marketable yield was positively and significantly correlated with leaves per plant, bulb diameter, weight of 20 bulbs and cloves per bulbat genotypic and phenotypic levels and negatively correlated with weight of 50 cloves per bulb (Singh *et al.* 2011). They also reported gross yield was positively and significantly correlated with plant height, neck thickness and negatively correlated with clove diameter, clove size index at genotypic and phenotypic levels, indicating that selection based on these traits will help in increasing the yield. Characters like number of leaves per bulb (0.151), bulb diameter (0.370), clove weight (0.131) and clove length (0.256) showed non-significant association with bulb weight while characters like clove diameter (-0.093) and number of bulbils per plant (-0.008) exhibited negative and non-significant association with bulb weight. It may be concluded from the correlations that,the traits, plant height, bulb diameter, bulb size index, weigh of 20 bulbs, clove diameter, clove size index and cloves bulb-1 are correlated to each other and helpful in increasing in the bulb yield as reported earlier by Dhar (2002) and Tsega *et al.* (2010). Sonkiya *et al.* (2012) reported the bulb yield (q/ha) was genotypically and phenotypically positively correlated with no of leaves, neck thickness, weight of 5 bulbs, no of cloves per bulb, length of cloves, equatorial diameter, days to maturity, TSS content and sulphur content.

Identification of ideal genotypes on the basis of stability of genotypes

An ideal genotype should perform stably with high yield. Such an ideal genotype is defined by having the greatest vector length of the high yielding genotypes and as represented by smallest concentric circle (**Fig. 3**). Such genotypes which rarely exist can be used as a reference genotype. A genotype is more desirable for traits under investigations, if it is placed closer to the ideal genotype. Thus, using the ideal genotype as the center, concentric circles were drawn to visualize the distance between each genotype and the ideal genotype. The genotypes PG-18 identified closer with reference to ideal genotype fell into the center of concentric circle, in terms of studied traits as compared to rest of the genotypes, indicating its high yield potential. In addition, PG-1, PG-19, PG-17, NRCWG-2, PG-32, JGL-96-198, PG-30 and NRCRG-1 located on the next concentric circle, can be regarded as desirable genotypes. However, genotypes G-1 and JG-03-263 circumventing ideal genotype circle situated at the third concentric circle were identified as average yielding genotypes.

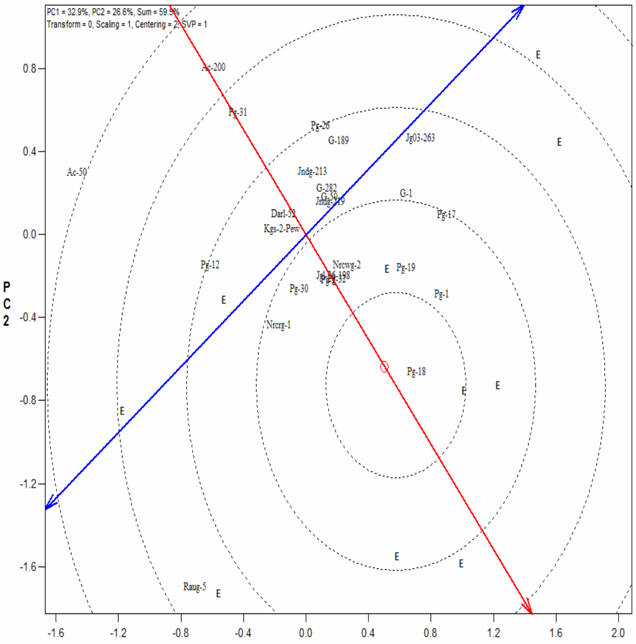

Fig. 3 Identification of ideal genotypes on the basis of stability of genotypes.

Ranking of genotypes based on overall performance

The test genotypes were compared diagrammatically for their stability using GGE biplot methodology of ranking genotypes on the basis of their performance (**Table 4**). To rank a genotype based on their performance, a line is drawn that passes through the biplot origin (**Fig. 4**). Based on the performance of the genotypes, best and poor performers were identified. The average tester coordination showed that among the best genotypes, PG-18 performed better and had good stability for all the characters. RAUG-5, PG-1, PG-19, PG-17, NRCWG-2, PG-32, JGL-96-198, NRCRG-1, G-1, PG-30 and JG-03-263 were average in their performance. Genotypes AC-50 and AC-200 are least stable genotypes for all characters. In contrast to this, genotypes like PG-31, PG-26, PG-12, G-189, JNDG-213, DARL-52, KGS-2-PEW, G-282 and JNDG-219 performed below average.

CONCLUSION

The present study led to the identification of geographically diverse garlic genotypes which carry desirable traits for productivity enhancement. Five genotypes namely PG-18, PG-17, PG-1, JG-03-263 and RAUG-5 having combination

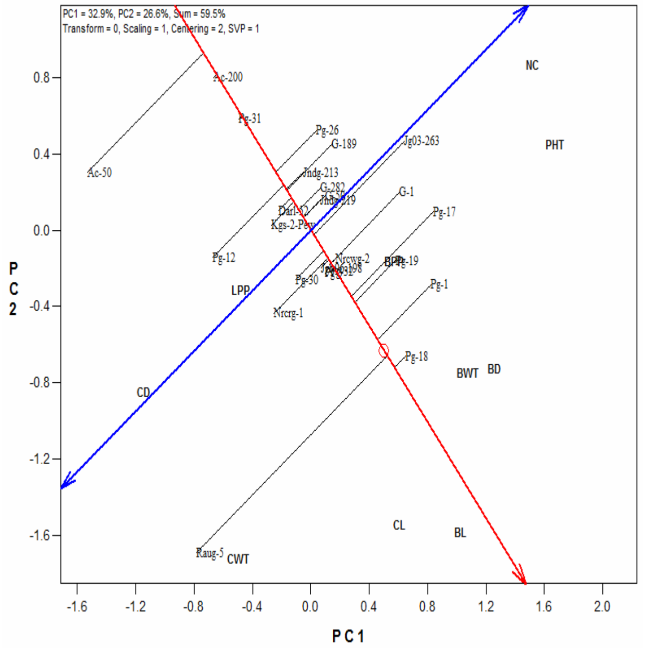

Fig. 4 Ranking of genotypes based on agro-morphological traits.

Table 4 Ranking of genotypes based on overall performance.

Row/Col	GGE Rank	GGE Distance	Mean
PG-18	1	0.1	0.779
PG-1	2	0.5	0.624
PG-19	3	0.6	0.162
PG-32	4	0.7	0.239
PG-3	5	0.7	-0.133
NRCWG-2	6	0.7	-0.026
JGL-96-198	7	0.8	0.235
PG-30	8	0.8	-0.007
NRCRG-1	9	0.9	0.24
PG-17	10	0.9	0.367
G-1	11	1	0.136
JNDG-219	12	1.1	-0.046
G-50	13	1.1	0.079
G-282	14	1.1	-0.169
KGS-2-PEW	15	1.2	0.063
DARL-52	16	1.2	0.167
JG03-263	17	1.2	0.164
JNDG-213	18	1.2	-0.155
G-189	19	1.3	-0.119
PG-12	20	1.4	0.067
PG-26	21	1.4	-0.39
RAUG-5	22	1.6	0.61
PG-31	23	1.7	-0.706
AC-200	24	2	-1.134
AC-50	25	2.4	-1.049

of positive traits were identified. Association studies indicated three traits viz. plant height, bulb length and number of cloves per bulb to be main determinants of bulb weight. Bulb length and clove length exhibited maximum positive correlation. Ranking on the basis of overall performance and stability analysis of genotypes for studied traits led to the identification of one genotype PG-18, having stable performance and high yield potential. In addition, PG-1, PG-19, PG-17, NRCWG-2, PG-32, JGL-96-198, PG-30 and NRCRG-1 were also identified as desirable genotypes for studied characters. In view of the preset study attention should be given on trait like plant height, bulb length and number of cloves per bulb for the improvement of bulb weight. The existence of considerable variation in the used garlic germplasm for bulb weight, clove weight, clove diameter and number of bulbils per plant can be exploited for the development of future cultivars. Genotypes PG-18, PG-1, PG-19, PG-32 and PG-3 should be used as one of the parents in the hybridization program, as they were having good combination of traits, depicted from their higher rank values.

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