

Unveiling of genetic variability in garlic (*Allium sativum* L.) genotypes grown in Pakistan

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Received: 22 June 2020; Accepted: 15 September 2020; Published online: 30 September 2020

Key Message: Unveiling of genetic variability in 4 garlic genotypes in comparison with two advanced lines was achieved. The results demonstrated that along with NARC G1, newly developed advance lines were also promising in several yield-related traits.

Abstract: Garlic (*Allium sativum*) is the second most widely cultivated *Allium* species grown in the world. But lack of development of improved varieties remains a major bottleneck in achieving higher yield. For varietal development programs, insufficiency of existing cultivars and genetic data are the major constraints. In the current study field trials for 3 consecutive years (2018 to 2020) were conducted to evaluate genetic variability among four different garlic genotypes in comparison with two newly developed advance lines. Based on 13 agro-morphological traits, garlic genotypes were evaluated in Randomized Complete Block design (RCBD) with three replications.

Highly significant variations were observed among the studied germplasms. Correlation analysis demonstrated that 100% germination had a highest positive correlation (0.970) with 20 plants dry weight, while the lowest positive correlation (0.816) was observed between flag leaf width and 100% germination. A highly negative correlation was observed between 100% germination and stem length. Principle component analysis (PCA) revealed differences among the genotypes. The PCA contributed more than 80% of variations for all the traits. From statistical data, it was inferred that NARC G1 performed better for almost all of the characteristics studied. Nevertheless, newly developed advance lines were also promising in several traits observed. © 2020 Department of Agricultural Sciences, AIOU

Keywords: Advance lines, *Allium sativum*, Garlic, Local Red, Variability

To cite this article: Rehman, M. A., Khattak, S. H., Inam, S., Rehman, N., Hussain, M., Ali, S., Shahid, M. M., & Khan, M. R. (2020). Unveiling of genetic variability in garlic (*Allium sativum* L.) genotypes grown in Pakistan. *Journal of Pure and Applied Agriculture*, 5(3), 53-64.

Introduction

Garlic (*Allium sativum* L.) botanically belongs to the Liliaceae family and *Allium* genus (Amarakoon & Jayasekara, 2017). Bulb of the garlic contains several cloves, which are used for reproduction (Liu et al., 2020). According to the FAO statistics 2018 data, garlic is the 4th most cultivated vegetable after tomato, potato and cassava. More than 20 edible *Allium* species are grown and consumed by humans all over the world including garlic, onion, leek, Japanese bunching onion and shallot (Van der Meer, 1994).

Garlic can be used as an antioxidant, antimicrobial, reduction of cardiovascular diseases, anti-cancer and anti-hypersensitive agent (Efiong et al., 2020; Wen et al., 2020) It is consumed as fresh form e.g. chopped, sliced or minced as well as dry powdered form (Gonzalez et al., 2009; Chen et al., 2018). It is beneficial to cure heart-related ailments (Aviello et al., 2009; Colín-González et al., 2012; Santhosha et al., 2013). Garlic is grown in diverse areas, grown in sunny and dry areas in a moderately humid and dry environment (Hanelt, 1990; Sayadi et al., 2020). Garlic

grows up to the height of 1.2 meters and its underground storage organ is called a bulb (Iciek et al., 2009).

China is the main producing and exporting country which contributes around 80% of the whole world raw garlic production. Other leading exporters are Argentina and Spain, while the leading importers are Pakistan, USA, Germany, Italy and France (Food and Agriculture Organization [FAO], 2018). Average garlic consumption is increasing day by day from the last decade. Its consumption increased from 1.6 kg to 3 kg per person per year (Gonzalez et al., 2009). Garlic consumers are increasing at a high rate in Pakistan but its production is not meeting the increasing demand (Baldwin et al., 2014). The world's average yield of garlic is 9.67 tons per hectare but the yield of Pakistan is hovering around 8.99 tons per hectare. Hence, a lot of foreign exchange is incurred on the import of garlic. The major reason for low yield is the lack of improved high yielding varieties that are adapted to local conditions (Wen et al., 2020). Genomic selection and long-read technology can be used for improving the yield-related traits of garlic varieties (Jung et al., 2019; Giorgetti et al., 2020). The situation is further aggravated due to less availability of arable land for garlic cultivation which is

only 8 thousand hectares. Therefore, the development of indigenous varieties of garlic with higher yield potential is inevitable. But very few studies have been conducted on the genetic variability of commonly grown cultivars grown in Pakistan. Therefore, a good comparison of genetic variability existing in available cultivars is a prerequisite. The aim of study was to unveil the genetic diversity by comparing the morphological traits among different garlic cultivars along with newly developed advance lines.

Materials and Methods

Acquisition of plant material

A total of 6 different garlic genotypes were used in the present study namely NARC-G1, Local White, Local Red, China White, Advance line1 and Advance line 2. The experimental materials (bulbs) of 6 garlic genotypes were collected from Bio Resources Conservation Institute (BCI), NARC, Islamabad.

Experimental site and experimental design

Geographically, the experimental site of the National Agricultural Research Centre, Islamabad falls under a humid subtropical climate and is located at 33.67° N latitude and 73.12° E longitude at an altitude of 507 meters above the sea level. The experiment was carried out in RCBD. All the experimental material was sown in three replications and the same experiment was repeated for 3 years to eradicate the risk of climatic factors influence. Cloves were sown in 3 lines with the line to line spacing of 11 inches and plant to plant distance of 6 inches.

Agronomical practices (Fertilizer, weeding and irrigation)

Fertilizer was applied according to the recommended dosage 125-75-60 (Sharma et al., 2015) for the garlic in 4 split applications. 1st dosage of fertilizer was applied in the preparation of soil to improve germination percentage. While the other 3 dosages were applied with consecutive irrigation applied. Weeding was done by hand every 15 days to remove the unwanted plant.

Morphological data collection

Data related to 13 different morphological traits were recorded from each year experiment, including days to 50% germination, days to 100% germination, plant height (cm), stem diameter (cm), stem length (cm), flag leaf length (cm), flag leaf width (cm), small leaf length (cm), small leaf width (cm), number of cloves per bulb, 100 cloves weight (g), bulb weight and 20 plants dry weight (g). The observation was recorded randomly from five different plants. The data of stem diameter, flag leaf width, small leaf width were measured in centimetres using a digital Vernier calliper. Plant height, stem length, flag leaf length and small leaf length were recorded using a measuring tape. The number of cloves per bulb was counted manually and digital balance was used to

determine the 100 cloves weight, bulb weight and 20 plants dry weight.

Statistical analysis

All the collected field data were subjected to various statistical tools. Statistics 8.1 and Minitab were used to compute ANOVA (analysis of variance) to test the significance and genotype x environment interaction and correlation as well. PCA analysis was done with the help of XL-STAT software to show the difference among the genotypes. Four separate PCA analyses were computed including the PCA analysis of each separate year and one combined 3 year PCA analysis. As a result, 2D graphs were generated to show the significant variation among the different genotypes in each year and combined 3 years as well.

Results

The morphological data analysis showed significant variations for all the traits observed. Table 2 demonstrates the ANOVA results for 3 years combined means data remarkably; the coefficient of variance (CV) was also less than 30 proving the reliability of the experiment. The coefficient of Variation as shown in Table 2 is a measure of the consistency of an experiment. It expresses the experimental error as a percentage of the mean. The higher the CV, the lower the precision associated with the comparison of treatment means. For field experiments, it must be in a range of 10-30%. The present study exhibits nearly 10% CV proving the precession of comparison of genotypes in terms of management and accuracy of data recording.

Data in Table 2 demonstrates that variation in the value of the observations due to the check i.e., Local Red is highly significant for the characteristics like plant height, stem diameter, stem length, flag leaf length, flag leaf width, small leaf length, small leaf width, number of cloves per bulb, 100 cloves weight, bulb weight, 20 plants dry weight, days to 50% germination and days to 100% germination. The line graph shown in Fig. 1 demonstrates the variations in five most important yield-related traits and among different genotypes. The plant height line is maximum in the Local White and minimum in NARC G1. Local White also exhibits more number of cloves as compared to the other genotypes while Advance line 2 and NARC G1 have the least number of cloves. Fig. 2 shows the variation in cloves shape and size among genotypes. In terms of Bulb Weight, NARC G1 bulbs are heavier while the Local Red had the minimum weight of the bulb. Fig. 3 represents different growth stages of Advance line 1. After 90 days of sowing variation in bulbs among different varieties are shown in Fig. 4. Days to 50 % germination line shows that the NARC G1 genotype took a higher number of days as compared to other genotypes while Local Red cloves germinated in less number of days. Days to 100 % germination line indicates that the NARC G1 genotype took more days as compared to other genotypes and all cloves of Local Red germinated in a minimum number of days.

A highly positive significant correlation was observed between the genotypes (Table 1) for 20 plants dry weight with 100% germination (0.970); flag leaf width with small leaf width (0.962); bulb weight with 100% germination (0.866); stem diameter with 100 cloves weight (0.841) and flag leaf length with small leaf length (0.835).

On the other hand, a highly negative correlation was shown for stem length with flag leaf width (-0.885); stem length with 50% germination (-0.893) and stem length with 100% germination (-0.845).

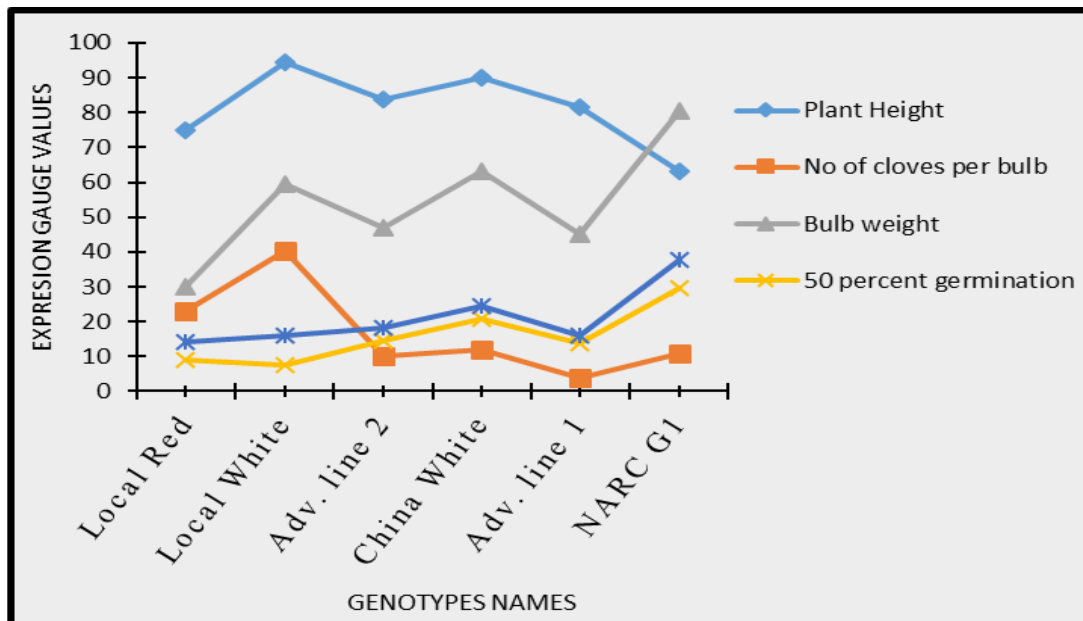


Fig. 1 Comparison of 5 yield related traits in all 6 genotypes. Values on the Y-axis indicate expression of different observation. Plant heigh (cm), Bulb weight (g), 50 percent germination (days) and 100 percent germination (days)

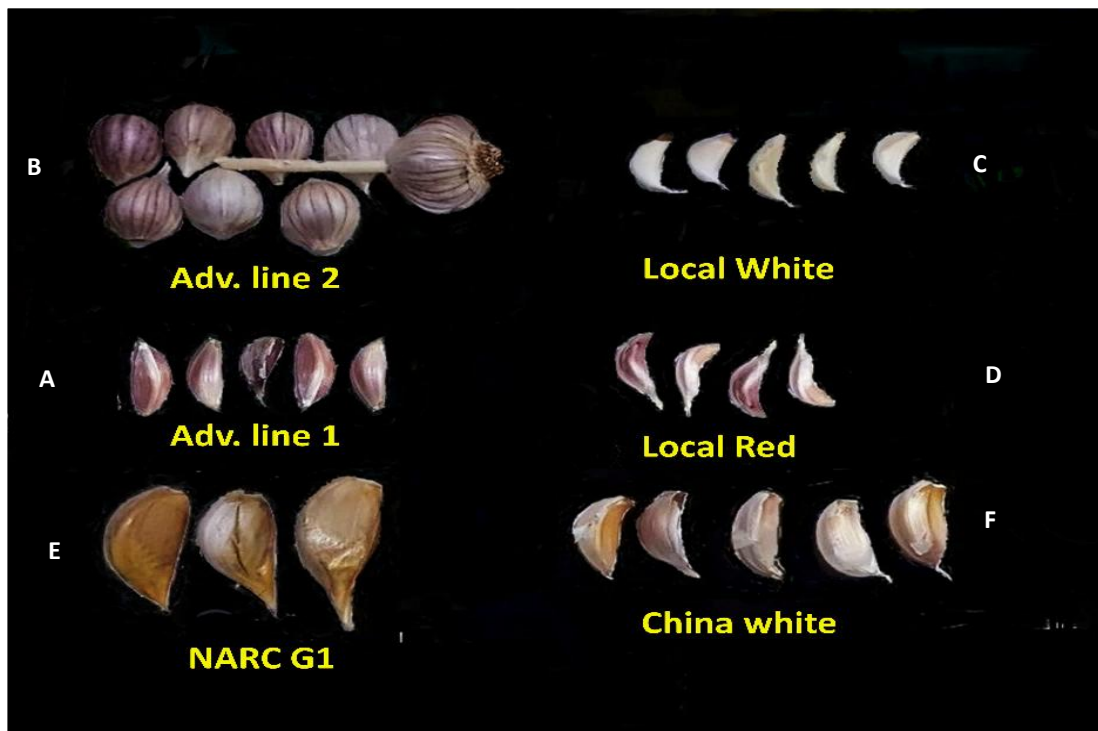


Fig. 2 Difference among the garlic cloves shape and size of various genotypes. (A) Advance line 1, (B) Advance line 2, (C) Local White, (D) Local Red, (E) NARC G, (F) China White

Table 1 Correlation of parameters for 3 combined years using XL-STAT software

| Variables | PH | SD | SL | FLL | FLW | SLL | SLW | NCPB | 100 CW | BW | 20 PDW | 50% G |
|-----------|--------|--------------|---------------|--------------|--------------|--------|--------|--------|--------------|--------------|--------------|--------------|
| PH | | | | | | | | | | | | |
| SD | -0.044 | | | | | | | | | | | |
| SL | 0.538 | -0.555 | | | | | | | | | | |
| FLL | 0.606 | 0.549 | -0.278 | | | | | | | | | |
| FLW | -0.404 | 0.795 | -0.885 | 0.466 | | | | | | | | |
| SLL | 0.672 | 0.609 | -0.163 | 0.835 | 0.267 | | | | | | | |
| SLW | -0.258 | 0.778 | -0.756 | 0.567 | 0.962 | 0.286 | | | | | | |
| NCPB | 0.570 | -0.539 | 0.554 | 0.231 | -0.445 | -0.090 | -0.218 | | | | | |
| 100 CW | -0.559 | 0.841 | -0.697 | 0.093 | 0.820 | 0.153 | 0.709 | -0.806 | | | | |
| BW | -0.489 | 0.193 | -0.726 | 0.235 | 0.727 | -0.216 | 0.754 | 0.062 | 0.322 | | | |
| 20 PDW | -0.666 | 0.328 | -0.801 | 0.121 | 0.820 | -0.262 | 0.797 | -0.193 | 0.553 | 0.956 | | |
| 50% G | -0.800 | 0.529 | -0.893 | -0.015 | 0.867 | -0.167 | 0.748 | -0.622 | 0.829 | 0.731 | 0.887 | |
| 100% G | -0.798 | 0.356 | -0.845 | -0.023 | 0.816 | -0.306 | 0.733 | -0.407 | 0.668 | 0.866 | 0.970 | 0.962 |

PH = Plant height; SD = Stem diameter; SL = Stem length; FLL = Flag leaf length; FLW = Flag leaf width; SLL = Small leaf length; SLW = Small leaf width; NCPB = Number of cloves per bulb; 100 CW = 100 cloves weight; BW = Bulb weight; 20 PDW = 20 plants dry weight; 50% G = 50% germination; 100% G = 100% germination; Values in bold show the significance level (alpha = 0.05); - ive value shows the negative correlations

Table 2 Correlation of parameters for 3 combined years using XL-STAT software

| Genotypes | DF | PH | SD | SL | FLL | FLW | SLL | SLW | NCPB | 100 CW | BW | 20 PDW | 50% G | 100% G |
|-------------|----|-----------|---------|-----------|---------|---------|----------|---------|-----------|-----------|-----------|-----------|-----------|-----------|
| Genotypes | 5 | 1067.15** | 0.90** | 1595.24** | 392.5** | 1.49** | 533.91** | 0.92** | 1235.56** | 1185848** | 2281.34** | 5432807** | 618.311** | 710.214** |
| Replication | 2 | 476.04 | 0.02385 | 3.53 | 5.667 | 0.00650 | 1.602 | 0.00576 | 0.10 | 1786 | 3.45 | 25391 | 7.881 | 1.547 |
| CV (%) | | 10.02 | 13.45 | 1.54 | 8.84 | 11.40 | 1.37 | 2.04 | 1.36 | 6.23 | 7.38 | 7.18 | 11.79 | 7.00 |

PH = Plant height; SD = Stem diameter; SL = Stem length; FLL = Flag leaf length; FLW = Flag leaf width; SLL = Small leaf length; SLW = Small leaf width; NCPB = Number of cloves per bulb; 100 CW = 100 cloves weight; BW = Bulb weight; 20 PDW = 20 plants dry weight; 50% G = 50% germination; 100% G = 100% germination; ** The mean difference is significant (highly significant at 0.01 level) based on the observed means; CV represents the coefficient of variance (range below 30%)



Fig. 3 Different growth stages of garlic Advance line 1 in the field. A: sowing in line B: the emergence of garlic C: three-leaf stage D: bulbing stage E: garlic plant at bulbing stage



Fig. 4 Different varieties at the bulbing stage after 90 days NARC G1, Advance line 1, China White Advance line 2, Local Red and Local White

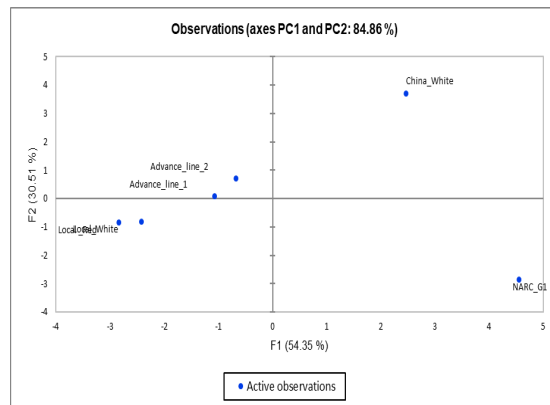


Fig. 5 Identification of genetic diversity on the bases of PCA analysis for experimental year 2018

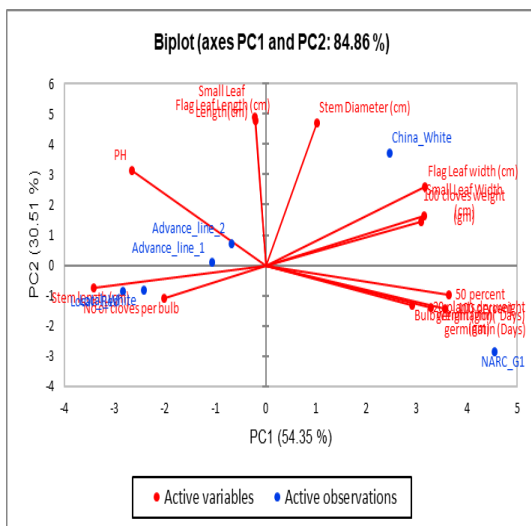


Fig. 6 Principal component analysis revealing Variation contribution by all the traits for experimental year 2018

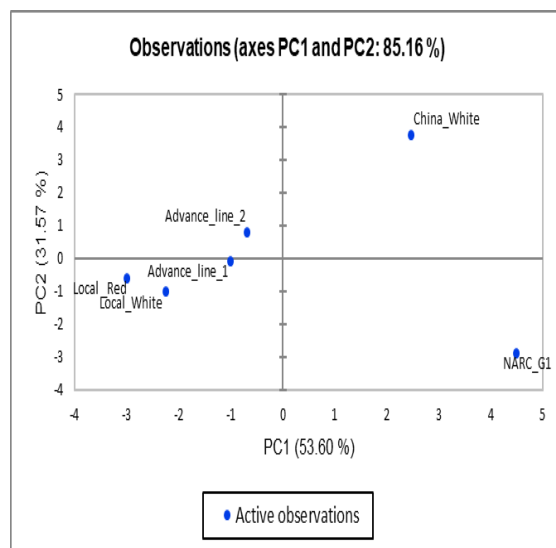


Fig. 7 Identification of genetic diversity on the bases of PCA analysis for experimental year 2019

Principal component analysis

Experimental year 2018 PCA analysis

PCA analysis showed diversity in different genotypes along with traits patterns (Fig. 6, Fig. 7 and Table 3). All the genotypes falling in the positive quadrant indicate the higher performance for those traits. On the other hands, genotypes categorized in the negative quadrants indicate lower performance for the traits. These analyses distributed the genotypes distinctively apart from advance lines which were grouped near to each other. PC1 contributed 54.35% of the total variations while PC2 contributed about 30.51%. China White was in the top right square, NARC G1 at the bottom right square while both advance lines were in the top left square and Local White and red were in the left bottom square.

Additionally, the biplot analysis indicated the interrelation among the genotypes as well as their stability and contribution toward an individual trait (Fakhar et al., 2019). In this regard, hierarchical clustering clearly distinguished three groups of genotypes according to their quality characteristics. The first group (right upper half of the graph) is formed by the genotypes China White with the highest values of stem diameter, flag leaf width, small leaf width and 100 cloves weight compared to other genotypes. The second group left side graph includes the genotypes advance lines and both Local White and red with the highest values of plant height, the number of cloves per bulb and stem length. The NARC G1 genotype is located in the last group i.e. lower right half of the graph.

Table 3 Contribution of the genotypic variation for the year 2018 PCA (%)

| Genotypes | PC1 | PC2 |
|----------------|--------|--------|
| Local Red | 19.087 | 3.005 |
| Local White | 13.903 | 2.777 |
| Advance line 2 | 1.085 | 2.148 |
| China White | 14.375 | 57.907 |
| Advance line 1 | 2.696 | 0.028 |
| NARC G1 | 48.855 | 34.135 |

Experimental year 2019 PCA analysis

Results dispensed all the genotypes apart from advance lines which have been grouped close to each other. Almost similar consequences had been acquired as the previous year with light modification of Advance line 1 that fell just below top left square. Furthermore, PC1 contributed 53.60% while PC 2 contributed 31.57% variation (Fig. 8; Fig. 9; Table 4). Similar groups were observed for the year 2019. The group at the right upper half of the graph includes genotypes China White with the highest values of stem diameter, flag leaf width, small leaf width and 100 cloves weight. The group at the left side of the graph consists of advanced lines and both Local White and Local Red with the highest values of plant height, number of cloves per bulb and stem length. The last group i.e. lower right half of the graph was only having genotype NARC G1. This group had the remaining parameters with the highest values apart from small leaf length and flag leaf length that were in-between groups one and two.

Table 4 Contribution of the genotypic variation for the year 2019 PCA (%)

| Genotypes | PC1 | PC2 |
|----------------|--------|--------|
| Local Red | 21.611 | 1.487 |
| Local White | 12.111 | 4.215 |
| Advance line 2 | 1.113 | 2.672 |
| China White | 14.564 | 57.798 |
| Advance line 1 | 2.465 | 0.023 |
| NARC G1 | 48.134 | 33.806 |

Experimental year 2020 PCA analysis

Parallel observations were reported for the year 2020 in the same manner as of previous years. The advance lines were clustered separately from other cultivars. Dissimilar results were obtained for Local Red and both advance lines as they were clustered near to each other proving the similarity between them. PC1 contributed 62.45% while PC 2 contributed 20.98% of the total variation (Fig. 10; Fig. 11; Table 5) for the biplot. The PCA analysis revealed a total of 4 groups for the year 2020. This year's results were a bit different as far as the grouping of genotypes is concerned. The first group consists of the genotypes China White with the highest values of flag leaf length and width, small leaf width, bulb weight and plant dry weight. The second group includes genotypes Local White with the highest values of small leaf length, plant height and number of cloves per bulb. The third group i.e. lower left half of the graph was having genotype Local Red and advance lines. This group had the highest value of stem length. While the last group had only NARC G1 genotype with height values of 100% germination and 50% germination.

Table 5 Contribution of the genotypic variation for the year 2020 PCA (%)

| Genotypes | PC1 | PC2 |
|----------------|--------|--------|
| Local Red | 21.184 | 4.607 |
| Local White | 10.192 | 58.393 |
| Advance line 2 | 1.999 | 12.932 |
| China White | 15.248 | 8.996 |
| Advance line 1 | 2.779 | 13.609 |
| NARC G1 | 48.597 | 1.463 |

Three combined experimental years (2018-2020) PCA analysis

To limit the environmental variations for 3 years, a combined PCA was carried out to show the diversity in the genotypes and to eradicate the errors in the analysis due to variable environmental conditions. The results show a significant difference in newly developed advance lines from the rest of the genotypes studied. Local White and Advanced line 2 was positioned in a top left quadrant of the PCA plot. Although advanced line 1 and Local Red were located in the bottom left quadrant yet they were close to each other in terms of distance proving similarity between them. Similarly, the remaining varieties behaved in the same manner as of all three years grouped near each other proving the similarity between them.

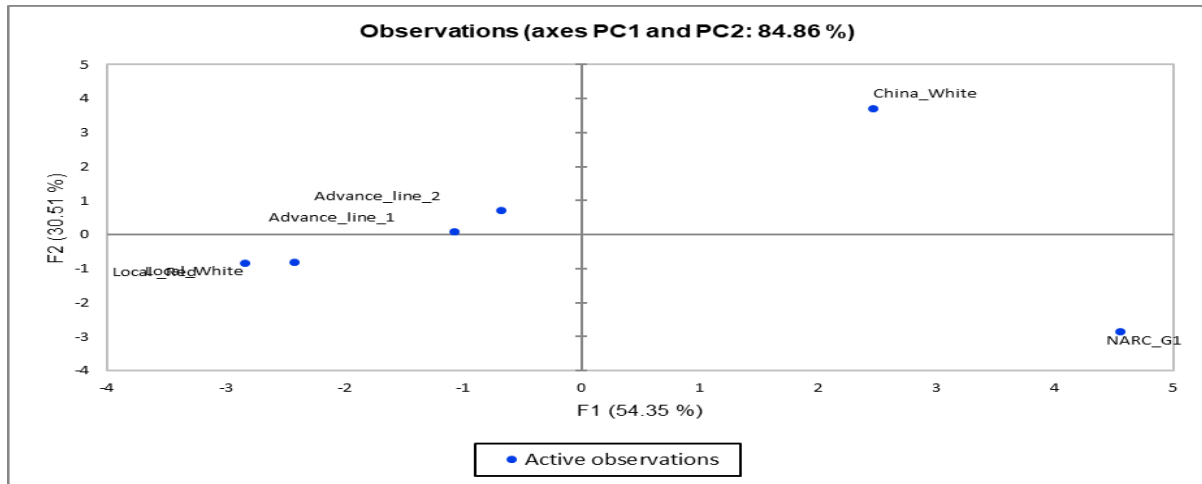


Fig. 8 Principal component analysis revealing variation contribution by all the traits for second experimental year 2019

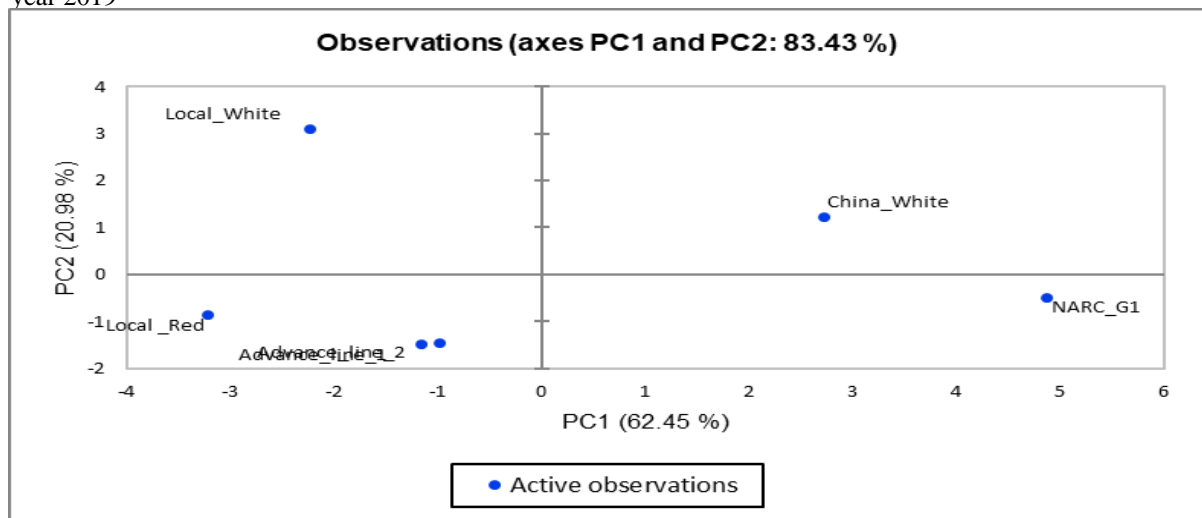


Fig. 9 Identification of genetic diversity on the bases of PCA analysis for experimental year 2020

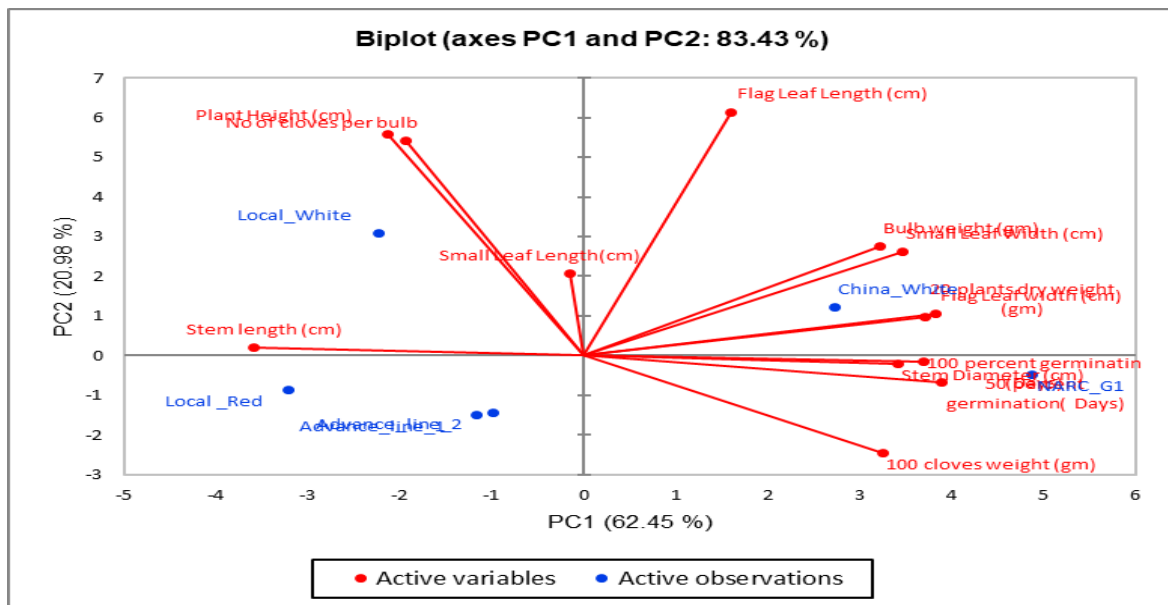


Fig. 10 Principal component analysis revealing variation contribution by all the traits for second experimental year 2020

Similar results were observed for advanced lines as for previous years. For a combined 3 years analysis, PC1 contributed 62.45% while PC 2 contributed 20.98% variation (Fig. 12; Fig. 13; Table 6). The 3 years combined PCA revealed 4 different groups. The first group (top right quadrant) includes the China White having the highest values for small leaf length, flag leaf length, stem diameter, flag leaf width, small leaf width and 100 cloves weight. The second group (top left quadrant) contains Local White and Advance line 1 for the higher values of plant height and number of cloves per bulb. The third group (bottom left quadrant) contains only the NARC G1 for the higher value of 100% germination and stem diameter. The fourth group (Bottom left quadrant) contains both Advance line 1 and Local Red. The Principal Scree plots of 3 consecutive years and a combined plot are given in supplementary file (Supplementary Fig. 1).

Table 6 Contribution of the genotypic variation for three years combined PCA (%)

| Genotypes | PC1 | PC2 |
|----------------|--------|--------|
| Local Red | 21.831 | 4.729 |
| Local White | 13.132 | 1.401 |
| Advance line 2 | 1.148 | 0.064 |
| China White | 18.773 | 56.876 |
| Advance line 1 | 2.280 | 0.602 |
| NARC G1 | 42.835 | 36.329 |

Grand mean of 3 years observations

The grand mean performance of three consecutive years serves as an important standard in evaluating the diversity among the genotypes. The result of the present investigation revealed that significant variations were observed for different traits. The grand mean performance and range of the 6 genotypes for all the thirteen traits are shown in Table 7. The average height of the different genotypes (Table 7) shows that Local white (107.3 cm) has the maximum height followed by the China White (90.8 cm), Local Red (87.6 cm) and the minimum value is of NARC G1 (53.8 cm). The data analysis regarding the mean values of stem diameter (Table 7) indicates that there is a significant difference observed among different genotypes. The maximum diameter of the stem was observed in China White (2.59 cm) followed by Advance line 1 (2.26 cm) and Advance line 2 (2.26 cm) while the minimum stem diameter was observed in Local Red (1.59 cm). The Average stem length data unveil that Local White (68.6 cm) has the maximum height followed by the China White (90.8 cm) and the minimum stem length was observed in NARC G1 (26.5 cm).

Flag leaf length data indicates that Local White (57.4 cm) has the maximum height followed by the China White (90.8 cm), and the minimum flag length was observed in the Local Red (49.7 cm). Flag leaf width data indicates that China White (2.93 cm) has the maximum width and the minimum width is of Local Red (1.82 cm).

The average small leaf length data of different varieties shows that China White (58 cm) has the maximum height and the minimum value is of NARC G1 (33.2 cm). Small leaf width data indicates that China White (2.41cm) has the maximum height and the minimum value is of Local Red (1.48 cm). The mean values show that a greater number of cloves bulb-1 was noted in variety Local white (37) followed by variety Local Red (22), China White (12), NARC-G1 (10) and Advance line 2 (10), respectively, while the least number of cloves bulb-1 was recorded in and Advance line 1.

The 100 clove weight data indicate that the maximum clove weight was noted in variety NARC-1 (913 g) followed by a variety of China White (896 g), Advance line 2 (746 g), Advance line 1 (703 g) and Local Red (114 g), respectively. Local White (92 g) gave the minimum 100 cloves weight. Mean weight computed from 5 different bulbs from each replication shows that variety NARC-1 (82g) had maximum value followed by variety China White (65 g), Local White (63 g) Advance line 2 (49 g) and Advance line 1 (48 g), respectively. Local Red (31 g) gives the minimum bulb weight. Days to 50 % and 100% germination shows that Local Red took fewer to attain followed by Local White, Advance line 2, Advance line 1 and China White, NARC G1 took around 38 days to accomplish 100 present germinations.

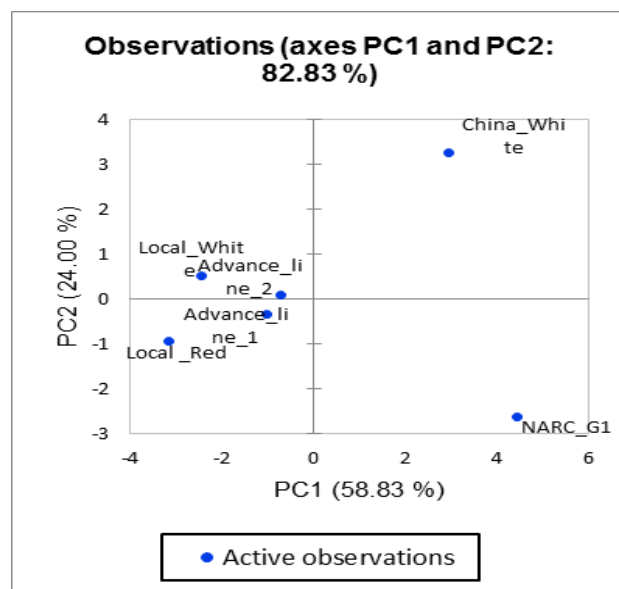


Fig. 11 Identification of genetic diversity on the bases of PCA analysis for 3 years combined experimental data

Table 7 Grand mean of three consecutive years recorded data 2018 to 2020

| Genotypes | PH | SD | SL | FLL | FLW | SLL | SLW | NCPB | 100 CW | BW | 20 PDW | 50% G | 100% G |
|----------------|-------|------|------|------|------|------|------|------|--------|----|--------|-------|--------|
| Replication 1 | | | | | | | | | | | | | |
| Local Red | 86 | 1.59 | 47.6 | 52.1 | 1.86 | 42.6 | 1.5 | 20 | 106 | 30 | 806 | 9 | 13 |
| Local White | 107.3 | 1.8 | 57.4 | 60.1 | 2.14 | 42 | 1.94 | 36 | 92 | 56 | 1333 | 6 | 16 |
| Advance line 2 | 85.2 | 2.13 | 50.8 | 54.2 | 2.21 | 43.2 | 1.82 | 9 | 720 | 47 | 1113 | 14 | 17 |
| China White | 90.8 | 2.52 | 26.8 | 67.8 | 2.93 | 56.3 | 2.39 | 12 | 868 | 59 | 1953 | 20 | 25 |
| Advance line 1 | 85.8 | 2.11 | 50.7 | 50.8 | 2.18 | 42 | 1.84 | 4 | 703 | 45 | 970 | 13 | 15 |
| NARC G1 | 72.4 | 2.06 | 26.5 | 49.7 | 2.86 | 33.2 | 2.22 | 10 | 913 | 82 | 2966 | 30 | 37 |
| Replication 2 | | | | | | | | | | | | | |
| Local Red | 87.6 | 1.65 | 48 | 52.9 | 1.82 | 42.4 | 1.48 | 22 | 106 | 31 | 833 | 9 | 14 |
| Local White | 95.6 | 1.87 | 56.5 | 60 | 2.1 | 42.2 | 1.9 | 37 | 88 | 54 | 1336 | 8 | 16 |
| Advance line 2 | 84.1 | 2.2 | 50.8 | 55.4 | 2.28 | 44.2 | 1.84 | 10 | 715 | 49 | 1146 | 15 | 18 |
| China White | 90.6 | 2.58 | 26.5 | 68.6 | 2.9 | 57.4 | 2.36 | 12 | 896 | 64 | 1826 | 22 | 23 |
| Advance line 1 | 82.8 | 2.26 | 50.7 | 52.2 | 2.19 | 42.8 | 1.84 | 4 | 670 | 48 | 966 | 14 | 17 |
| NARC G1 | 63 | 2.08 | 25.7 | 51.9 | 2.7 | 33.5 | 2.22 | 10 | 858 | 80 | 2786 | 29 | 38 |
| Replication 3 | | | | | | | | | | | | | |
| Local Red | 86 | 1.68 | 48.4 | 53 | 1.9 | 42.8 | 1.49 | 20 | 114 | 31 | 826 | 8 | 14 |
| Local White | 80.6 | 1.85 | 57.8 | 60.8 | 2.11 | 42 | 1.96 | 34 | 87 | 63 | 1570 | 7 | 15 |
| Advance line 2 | 81.4 | 2.17 | 51 | 55.5 | 2.29 | 43.8 | 1.84 | 9 | 746 | 46 | 1083 | 14 | 18 |
| China White | 88.6 | 2.59 | 27.4 | 67.6 | 2.9 | 58 | 2.41 | 10 | 850 | 65 | 1886 | 19 | 24 |
| Advance line 1 | 75.8 | 2.17 | 51.6 | 51.4 | 2.2 | 42.6 | 1.87 | 4 | 651 | 45 | 1006 | 13 | 15 |
| NARC G1 | 53.8 | 2.06 | 27.2 | 51.8 | 2.8 | 33.2 | 2.29 | 10 | 835 | 77 | 2973 | 29 | 37 |

PH = Plant height; SD = Stem diameter; SL = Stem length; FLL = Flag leaf length; FLW = Flag leaf width; SLL = Small leaf length; SLW = Small leaf width; NCPB = Number of cloves per bulb; 100 CW = 100 cloves weight; BW = Bulb weight; 20 PDW = 20 plants dry weight; 50% G = 50% germination; 100% G = 100% germination; This table shows the performance comparison of newly developed advance lines with different genotypes; Plant height, stem diameter, stem length, flag leaf length, flag leaf width, small leaf length, and small leaf width were measured in cm; 100 cloves weight, bulb weight, 20 plants dry weight were taken in g; 50 percent germination and 100 percent germination were taken in days; China White was promising in almost all the traits; Newly developed Advance line 1 and Advance line 2 performed better than its parent Local Red.

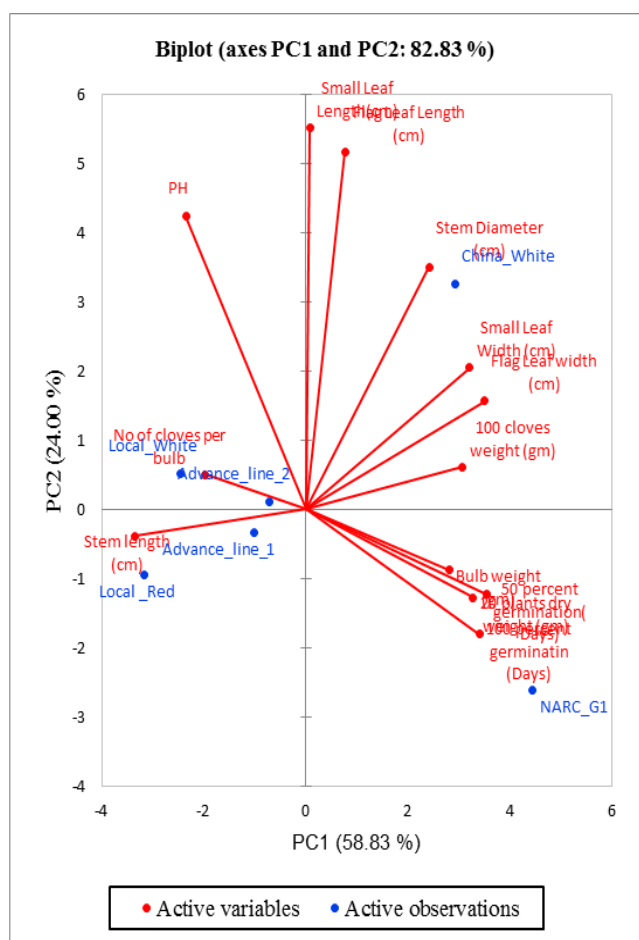


Fig. 12 Principal component analysis revealing, variation contribution by all the traits for three years combined experimental data

Discussion

With an increase in the consumption of garlic worldwide, plant breeders are striving to develop new varieties to cope with the increasing demand and to fulfil the consumer requirement. Two newly developed advance lines developed from Local Red progenies by eight years of mass selection (best performance across various yield-related traits). Selected two advance lines performed differently and better than their parental lines. The results showed highly significant variations between the studied genotypes. Garlic is only reproduced by vegetative propagation and yet displays considerable morphological variation within and between genotypes (Kumar et al., 2019). To broaden the breeding program genetic diversity between individuals is very important for the improvement of garlic yield (Kumar et al., 2019). The outcomes of the present study are in agreement with the conclusion of (Yuqiu et al., 2019) that the emergence and growth of garlic can be different as it depends on the planting time and genotype. The present study shows that the China White has the maximum yield in terms of dry bulb weight while the minimum yield is shown by the genotype Local White. Sharma et al. (2018) concluded the research

findings that the highest plant density and cloves germination time can lead to loss in the yield and the lowest bulb diameter and bulb weight. The correlation analysis showed the negative correlation between the bulb weight and days to 50 % or 100 % germination.

Fakhar et al. (2019) also studied the correlation among different genotypes of garlic and found the positive correlation between the plant height and leaf length. A similar positive correlation was observed in the present studies (Khan et al., 2018) proposed that stem diameter, yield and bulb weight is negatively correlated with plant height. The current study also shows a similar variation in the observed data. Evaluation of agronomic traits revealed significant variations in the garlic genotype as observed, the China White was performing best in almost all the traits observed during the studies and they have the highest yield potential. Although the newly developed advance lines were also performing better than its parent genotype Local Red and also as compared to exotic genotype Local White. Raja et al. (2017) also highlighted that different genotypes show great variability in traits in diverse environmental conditions. In this study both advance lines have the high yield potential, the bulb weight was highest compared to the Local Red and Local White genotypes.

Principal components analysis (PCA) results show a significant difference in newly developed advance lines from the rest of the genotypes studied. Local White and advanced line 2 were placed in a top left quadrant of the all the PCA plot as it is the high yielding genotype. Although advanced line 1 and Local Red were in the bottom left quadrant yet they were close to each other in terms of distance proving similarity between them as advance lines are developed from Local Red progeny. Similarly, the remaining varieties behaved in the same manner as of all three years grouped near each other proving the similarity between them. Similar findings were reported by Kumar et al. (2019). Although, the present studied PCA results are slightly different from his study results as they both principle components contributed 58.73% to the total variations while the present study showed more than 80% contribution for all and combined PCA. The differences could be due to different breeding material and focusing on different parameters.

Testing and evaluation of the genotypes geographically from different regions of the world can help the breeders to develop new high yielding genotypes. Assessment of their variability is the first step for the development and improvement of existing genotypes. That will also help the breeders for the improvement of varieties; yield-related characteristics must be studied to evaluate those genotypes. This study will help the farmers to use high yielding varieties to get more productivity out of their field and also breeder to utilize those genotypes as a parent plant in the development of new varieties.

Conclusion

Genetic variability with regards to yield exists in 4 tested genotypes. Along with NARC G1, newly developed Advance lines performed better in different observed traits as compared to its parent variety.

Authors Contribution Statement: Muhammad Ramzan Khan conceptualized the idea, supervised the research study, assembled the results, and wrote the manuscript. Malik Attique ur Rehman, Nazia Rehman and Safeena Inam carried out the field trials and collected the data. Sahir Hameed Khattak, Shaukat Ali and Muzamil Hussain analyzed the data and interpreted the results. Muhammad Munir Shahid provided the guidance and statistical software.

Conflict of Interest: The authors declare no conflict of interest

Acknowledgment: Sincere appreciation and gratitude to Malik Muhammad Yar, a progressive farmer of Bhera, Sargodha for financial support and useful comments on agronomic practices of garlic trials.

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