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Morphological, Reproductive and Biochemical Diversity in Luffa (*Luffa aegyptiaca*) Cultivars

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ABSTRACT

Cucurbitaceae comprises a variety of vegetables with global economic importance. However, many of these vegetables remain unexplored regarding their genetic diversity for morphological and biochemical traits. The present research was conducted to classify, categorize, characterize, and conserve the available luffa gourd germplasm in Pakistan. Sixteen luffa gourd cultivars were statistically analyzed using a randomized complete block design (RCBD) along with PCA and cluster analysis. Morphological and reproductive traits were studied along with biochemical contents. The interspecific diversity of the samples was investigated by cluster analysis and similarity matrix of the entries. Principal coordinate analysis was performed to understand the variation among landraces and correlation matrix to observe patterns among the variables. Considerable morphological variations occurred in vegetative characteristics (i.e., growth habit, leaf, and stem shape, leaf margin, and intermodal length), reproductive traits (fruit length, width, and weight, fruit count per plant and yield per plant), and biochemical attributes, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and protein. Cluster analysis depicted two main groups of 9 and 7 genotypes while each was further divided into two and three subgroups, respectively.The results of phenotypic correlation, exhibited fruit yield had significant positive correlation with fruit weight (0.843**) as well with the number of fruits per plant (0.80**). However, principal component analysis depicted a high variation (42.05% and 28.80% of total variation) based on mineral composition, reproductive and biochemical traits. The results confirmed a substantial inter/intraspecific genetic diversity and broad relationships among luffa gourd genotypes. Thus, the study serves as a foundational framework for breeding activities, providing valuable genetic descriptors for luffa gourd cultivation. It offers a roadmap for researchers to select genotypes with high genetic variation, thus paving the way for breeding efforts to develop superior hybrids and varieties.

Introduction

Luffa gourd (*Luffa aegyptiaca* L.) (2n = 2x = 26) belongs to the Cucurbitaceae family (Jiang et al., 2014) and is widely cultivated in subtropical regions in different countries throughout the world (Demir, 2006). Enriched with vitamins and fibers, it provides higher levels of lignin and hemicelluloses (Wanf et al., 2019). The fruit is

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frequently used in gastric, antipyretic, and anthelmintic cases as a traditional treatment against ailments (Etim et al., 2018). Its leaves possess saponins, which directly cause relaxing effects on anoxia and fatigue, with enhanced immunological activity (Shendge and Belemkar, 2018). Luffa has applications as bath and utensil cleaning sponges. It is also used in cosmetic industries and as an adsorbent for heavy metals in wastewater (Phan et al., 2015).

Breeders and producers can achieve sustainable agriculture by gathering, analyzing, presenting, and utilizing genetic resources (Pathirana and Carimi, 2022). Notably, significant advancements have happened in plant germplasm conservation in recent decades. However, the proliferation of germplasm collections has frequently posed challenges to accessibility and utilization in plant breeding and research. The complexities of exploiting extensive germplasm for research purposes prompted researchers to evaluate, characterize, and establish the best genotypes. These collections resulting from the characterization cannot replace the existing germplasm. They serve as manageable selections designed to represent the genetic diversity in larger germplasms (Salgotra and Chauhan, 2023). Two major luffa species are *Luffa aegyptiaca* and *Luffa cylindrica*. They abundantly grow worldwide, particularly in Asia (Maamoun et al., 2019). Both species differ in terms of qualitative as well as quantitative characters such as number and weight of fruits (Prakash et al*.,* 2013), uniformity in size and thickness of fruit, bitterness or sweetness (Som et al*.,*2020), sex ratio (male: female), earliness of flowering, fruit setting and maturity, fibrous or non-fibrous fruit at edible stages along with resistance against viral and fungal diseases (Singh et al., 2017).

Previous research evaluated genetic variation in luffa gourd germplasm. However, determining the relationship between these species is imperative for effective utilization, exploitation, or suggesting breeding programs in different aspects of Luffa gourd (i.e., in traditional medicine). Although molecular tools have offered attractive prospects for genetic characterization, morphological studies are worthy for initial characterization and germplasm classification (Agdagwa and Nadukwa, 2004; Sudre et al., 2010). The characterization was either through genetic or morphological methods and resulted in large data sets, though difficult to interpret and understand. Consequently, statistical tools assist in quantifying variations in a germplasm collection, encompassing morphological, physiological, and molecular traits. This approach enables researchers to understand the overall diversity of the collection and identify potentially valuable genotypes (Mondal et al., 2023). Among these statistical tools, cluster analysis and principal component analysis (PCA) help to identify groups within the collection with similar characteristics, uncovering hidden patterns and relationships between different accessions (Wendwessen, 2023).

Variability in cucurbitaceous crops occurs in the form of landraces, traditional cultivars, wild relatives, and related non-edible wild-weedy species (Chomicki et al., 2019). In Pakistan, insignificant amounts of research have dealt with genetic improvements in luffa gourd by collecting diverse accessions in the germplasm to study their morphological characterization and evaluate coefficients of variation. The improvement of luffa gourd through successive selection for desirable traits has decreased the genetic diversity of luffa cultivars. Thus, this study considered local and hybrid luffa gourd germplasm by characterizing morphological, reproductive, and qualitative features. This research also aimed to identify sustainable, high-quality accessions in the germplasm for varietal development and recommend cultivation advice to farmers.

Material and Methods

The research was carried out in the summer season at the Vegetable Farm of the Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. Sixteen luffa gourd cultivars were collected from various sources (Table 1). The cultivars were sown outdoors, with a planting pattern of 3.5 × 2 sq ft.. All agronomic practices, i.e., weeding, hoeing, staking, and trellising were performed accordingly.

Vegetative and reproductive traits

Vegetative and reproductive growth attributes were assessed according to a method described by Abdel- Mawgoud et al. (2010), following a descriptor outlined by Joshi et al. (2004).

Yield related traits

Fruits per plant, fruit yield per plant (g), fruit length (cm), fruit width (cm), and fruit weight (g) were calculated after harvest (Saleem et al., 2013).

Seed related traits

Seed count per fruit, seed color (black, gray, brown, and white), 100-seed weight (g), seed length (mm) and seed width (mm) were calculated after harvest according to descriptions by Joshi et al. (2004) and Weydert and Cullen (2010).

Qualitative traits
Leaf chlorophyll content (Coste et al., 2010), leaf mineral (NPK) content (Weydert and Cullen, 2010), leaf enzymatic assay on catalase and peroxidase (Liu et al., 2009), and superoxide dismutase activity (Stagner and Popovic, 2009) were also evaluated.

Statistical analysis
The current study comprised a randomized complete block design (RCBD) under open-field conditions. With sixteen treatment groups and three replications, the experiment had three plants per replication. Statistix 8.1 software performed ANOVA under RCBD along with the Least Significance Difference (LSD) test ($P<0.05$) to determine significant differences among cultivars (Steel et al., 1997). Principal Component Analysis and Cluster Analysis ran via the statistics program WINDOSTAT 9.2.

Table 1. Luffa gourd genotypes and their sources.

Coding	Variety name	Source*	Coding	Variety name	Source*
V_1	All Green	1	V_9	Sponge gourd hybrid	4
V_2	Advanta 1103	2	V_{10}	Ridge gourd 619	3
V_3	Dilpasand	1	V_{11}	Sponge gourd GARIMA	3
V_4	Sponge gourd Local	1	V_{12}	Sponge gourd AVANTI	3
V_5	Ridge gourd 625	3	V_{13}	Sponge gourd HDH 245	3
V_6	Sponge gourd 1102	2	V_{14}	Jaipuri Long	3
V_7	Sponge gourd 2	2	V_{15}	Nutech India	3
V_8	Sponge gourd 3	2	V_{16}	Gloob F_1 hybrid	3

*1- AARI Faisalabad, 2- ICI-Pakistan Ltd, 3- Global Seeds Faisalabad, 4- Pak Agri. Seeds, Gujranwala.

Results
Morphological characterization of luffa gourd revealed considerable phenotypic variation. The growth habit of luffa varied significantly, with intermediate growth being prominent, followed by bushy and prostrate growth, respectively. A similar pattern occurred in almost all genotypes for leaf shapes and leaf margins. Leaf lobes were in uniform shape with smooth margins having intermediate to absent leaf lobes, dominant with orbicular shapes and dented margins. An exception appeared in sponge gourd (cv. AVANTI), where an ovate leaf shape occurred with shallow leaf lobes. Dorsal and ventral leaf pubescence also proved highly polymorphic among different genotypes as absent to low, with high to intermediates dorsal and ventral leaf pubescence, respectively. Stem shapes appeared diverse from round to angular among genotypes. Maximum similarity was observed in tendrils as they were present in all genotypes (Table 1).
Fruit shapes differed from elongate to elliptical, whereas variations occurred as elliptical, slim, and tapered fruits. Fruit ribs were prominently absent in approximately all genotypes, with little exceptions in the case of “Ridge gourd 619 and 625”. Flower colors were morphologically important to pollinators among floral characteristics, especially when forecasting pollination as a prelude to yield. The flower color proved highly homozygotic, with an innate “Yellow” color as prominent (Tables 2 and 3).
Fruit length is also one of the essential factors in determining fruit yield and marketability. The results showed highly significant differences in all genotypes (Fig. 1a). V_{16} (Gloob F1 Hybrid) had the highest fruit length (25.62 cm), and the minimum (18.90 cm) appeared in V_{15} (Nutech India). Likewise, noteworthy variations were observed in luffa fruit diameter as values ranged between 7.81 cm and 10.96 cm (Fig. 1b). The maximum diameter (10.96 cm) was observed in V_7 (Sponge gourd-2) and the minimum (7.81 cm) in V_3 (Dilpasand). Fruit diameter is directly proportional to ultimate luffa yield (Fig. 1b).

Table 2. Morphological characteristics of growth habit, leaf, and stem in select luffa genotypes.

Genotypes	Growth habit	Leaf shape	Leaf margin	Leaf lobes	Dorsal Leaf pubescence	Ventral Leaf pubescence	Stem shape	Tendril
All Green	Intermediate	Reniform	Smooth	Intermediate	Absent	Low	Round	Present
Advanta 1103	Intermediate	Reniform	Dented	Intermediate	Absent	Intermediate	Angular	Present
Dilpasand	Bushy	Orbicular	Smooth	Intermediate	Low	High	Angular	Present
Sponge gourd Local	Intermediate	Orbicular	Smooth	Absent	Absent	Intermediate	Round	Present
Ridge gourd 625	Intermediate	Reniform	Dented	Intermediate	Absent	Low	Round	Present
Sponge gourd 1	Intermediate	Orbicular	Dented	Absent	Intermediate	High	Angular	Present
Sponge gourd 2	Intermediate	Orbicular	Dented	Absent	Low	High	Angular	Present
Sponge gourd 3	Intermediate	Orbicular	Dented	Absent	Low	High	Round	Present
Sponge gourd hybrid	Intermediate	Reniform	Dented	Shallow	Absent	Intermediate	Round	Present
Ridge gourd 619	Prostrate	Orbicular	Smooth	Absent	Low	High	Angular	Present
Sponge gourd GARIMA	Bushy	Orbicular	Smooth	Intermediate	Absent	High	Round	Present
Sponge gourd AVANTI	Bushy	Ovate	Smooth	Shallow	Low	Intermediate	Round	Present
Sponge gourd HDH 245	Intermediate	Orbicular	Dented	Shallow	Absent	Medium	Round	Present
Jaipuri Long	Bushy	Reniform	Dented	Intermediate	Absent	Medium	Angular	Present
Nutech India	Intermediate	Reniform	Dented	Shallow	Low	High	Intermediate	Present
Gloob F1 hybrid	Bushy	Reniform	Smooth	Absent	Absent	High	Round	Present

Table 3. Morphological characteristics of flower, fruit, and seed color in select luffa genotypes.

Genotypes	Fruit shape	Fruit ribs	Stem end fruit shape	Blossom end fruit shape	Flower colour	Friut colour	Seed Colour
All Green	Oblong blocky	Absent	Pointed	Rounded	Yellow	light green	Black
Advanta 1103	Elliptical	Absent	Rounded	Flattened	Yellow	Green	Black
Dilpasand	Elongate elliptical	Absent	Rounded	Rounded	Yellow	Green	Black
Sponge gourd Local	Elongate slim	Absent	Rounded	Rounded	Yellow	Green	Brown
Ridge gourd 625	Elongate Tapered	superficial	Pointed	Rounded	Yellow	light green	Black
Sponge gourd 1	Elongate Tapered	Absent	Pointed	Flattened	Yellow	Green	Black
Sponge gourd 2	Elliptical	Absent	flattened	Rounded	Yellow	dark green	Black
Sponge gourd 3	Rounded	Absent	Rounded	Flattened	Yellow	light green	Black
Sponge gourd hybrid	Elongate elliptical	Absent	Rounded	Flattened	Yellow	Green	Black
Ridge gourd 619	Elongate elliptical	superficial	Pointed	Depressed	Yellow	dark green	Black
Sponge gourd GARIMA	Eliptical	Absent	Pointed	Pointed	Yellow	Green	White
Sponge gourd AVANTI	Eliptical	Absent	Rounded	Pointed	Yellow	Green	White
Sponge gourd HDH 245	Elongate slim	Absent	Rounded	Pointed	Yellow	dark green	Black
Jaipuri Long	Elongate slim	Absent	Pointed	Pointed	Yellow	light green	Black
Nutech India	Elongate elliptical	Absent	flattened	Pointed	Yellow	Green	Black
Gloob F1 hybrid	Elongate elliptical	Absent	flattened	Pointed	Yellow	dark green	Black

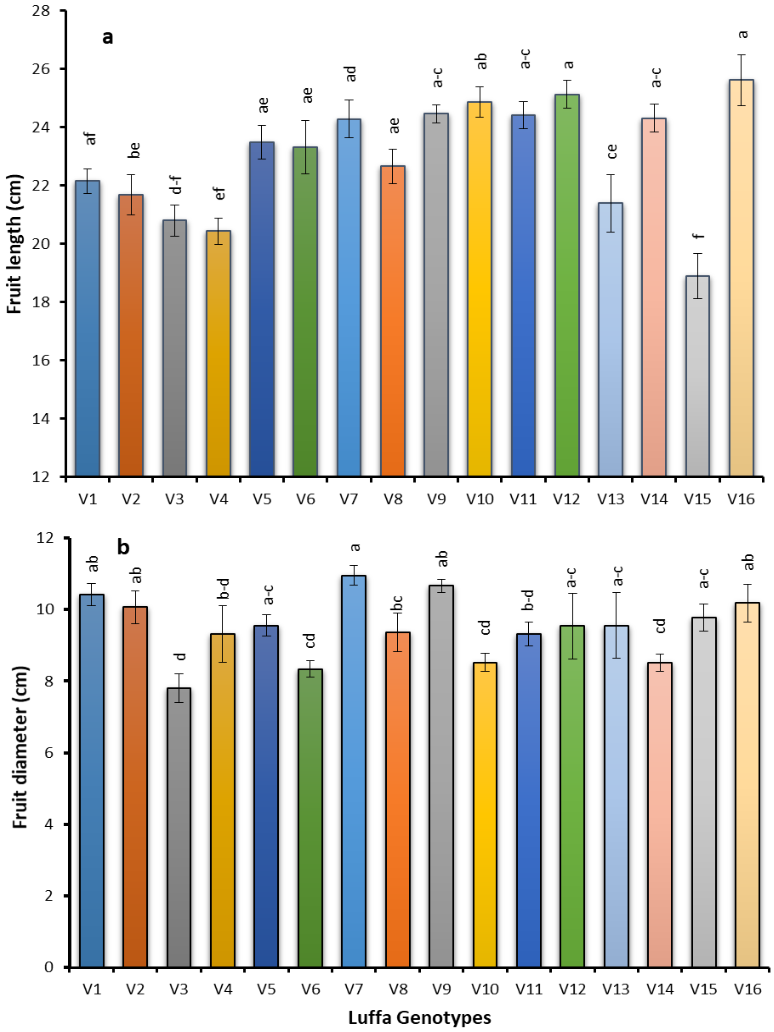


Fig. 1. Histogram for fruit length (a) and fruit diameter (b) of various luffa genotypes. Error bars with standard deviation appear with relevant letters. Mean values with similar letters are statistically non-significant (P≤0.05).

Fruit weight is an important aspect of productivity. Significant differences between cultivars were found in luffa fruit weight, with values ranging between 65.31 and 88.30 g (Fig. 2a). The results showed that V6 (Sponge gourd 1102) had relatively higher fruit weight (88.30 g), followed by V4 (Sponge gourd local) (83.33 g). The minimum fruit weight was observed in V_{12} (Sponge gourd Avanti) (65.31 g). Significant variations occurred in fruit count per plant and fruit yield per plant. The V_6 (Sponge gourd 1102) had a maximum of 18.22 fruits per plant, with an average fruit yield of 1609.0 g per plant. The minimum fruit count was 14.22 per plant, with an average fruit yield of 928.9 g in V_1 (All Green), respectively (Figs. 2b and 3a).

Among vegetative growth characteristics, internodal length is a significant attribute that signifies plant health, growth rate, and ultimate crop vigor. The mean values of internodal distance regarding genotypes appear in Figure 3b. In particular, V_5 (Ridge Gourd 625) developed the maximum internodal distance (11.21 cm), followed by V_2, V_9, and V_{10}, whereas the minimum value occurred in V_8 and V_{14} (6.11 cm) (Fig. 3b). Significant differences appeared similarly in the case of petiole length, where V_3 (Dilpasand) showed maximum petiole length (8.20 cm), whereas V_2, V_{13}, and V_{14} had minimum petiole length (6.40 cm) (Fig. 4a). The first fruit harvest for vegetable use showed significant outcomes (Figure 4b). The minimum number of days to first harvest (48 d) was observed in V_6 and V_9, while the maximum number of days to first harvest (63 d) was in V_3. However, the number of days to last harvest depended on how long a genotype could continue to yield. The following genotypes had a long-lasting ability for fruit yield (93 days): V_{12}, V_{13}, and V_{15}, which stands in contrast to V_2, which only yielded fruits for 66 d (Fig. 5a).

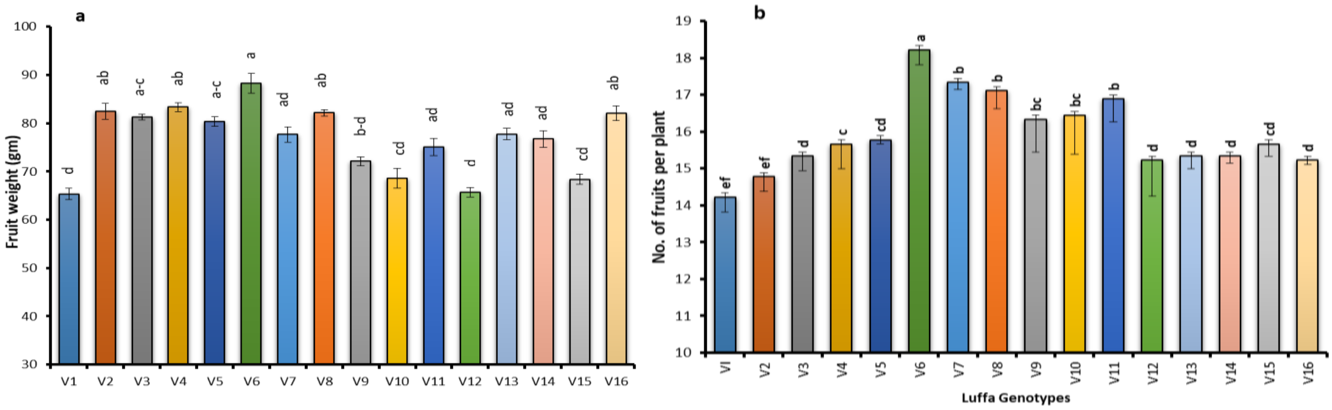


Fig. 2. Histogram for fruit weight (a) and number of fruits per plant (b) in various luffa genotypes. Error bars with standard deviation show relevant letters. Mean values with similar letters are statistically non-significant (P≤0.05).

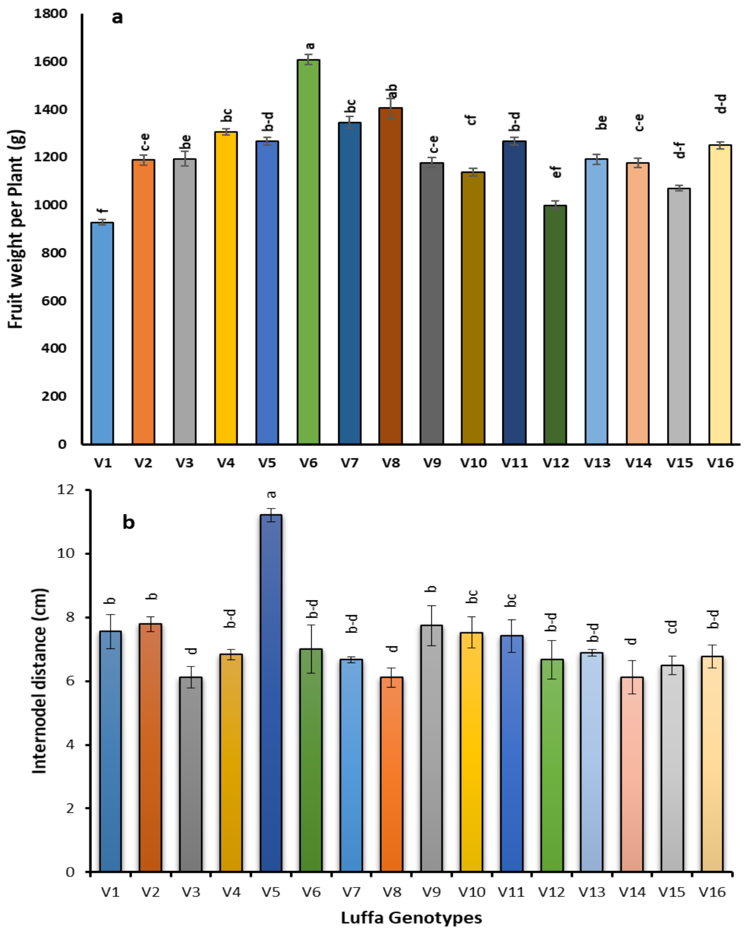


Fig. 3. Histogram for fruit weight per plant (a) and internodal distance (b) of various luffa genotypes. Error bars with standard deviation show relevant letters. Mean values with similar letters are statistically non-significant (P≤0.05).

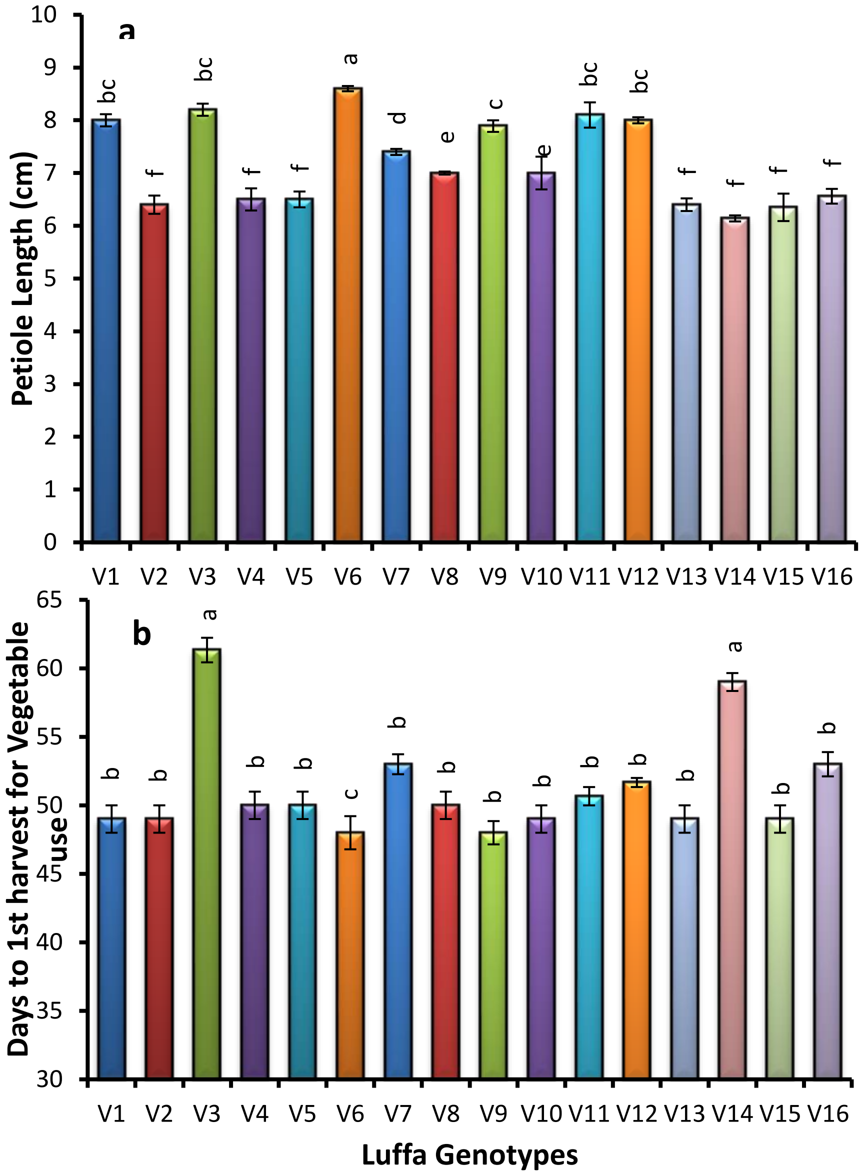


Fig. 4. Histogram for petiole length (a) and days to first harvest for vegetable use (b) of various luffa genotypes. Error bars with standard deviation show relevant letters. Mean values with similar letters are statistically non-significant (P≤0.05).

The mean values of seed length and genotypes appear in Figure 5b. V_{16} had maximum seed length (13.32 mm), followed by V_5, V_{14} and V_{13} while the least was recorded in V_1 (10.07 mm) (Fig. 5b). Seed width from different luffa gourd genotypes differed significantly. Genotype V_9 recorded maximum width (8.04 mm) while minimum width (6.50 mm) occurred in V_{14} (Fig. 6a). Similar results were observed regarding seed count per fruit and 100-seed weight, where significant genetic variation occurred among genotypes (Figs. 6b and 7a).

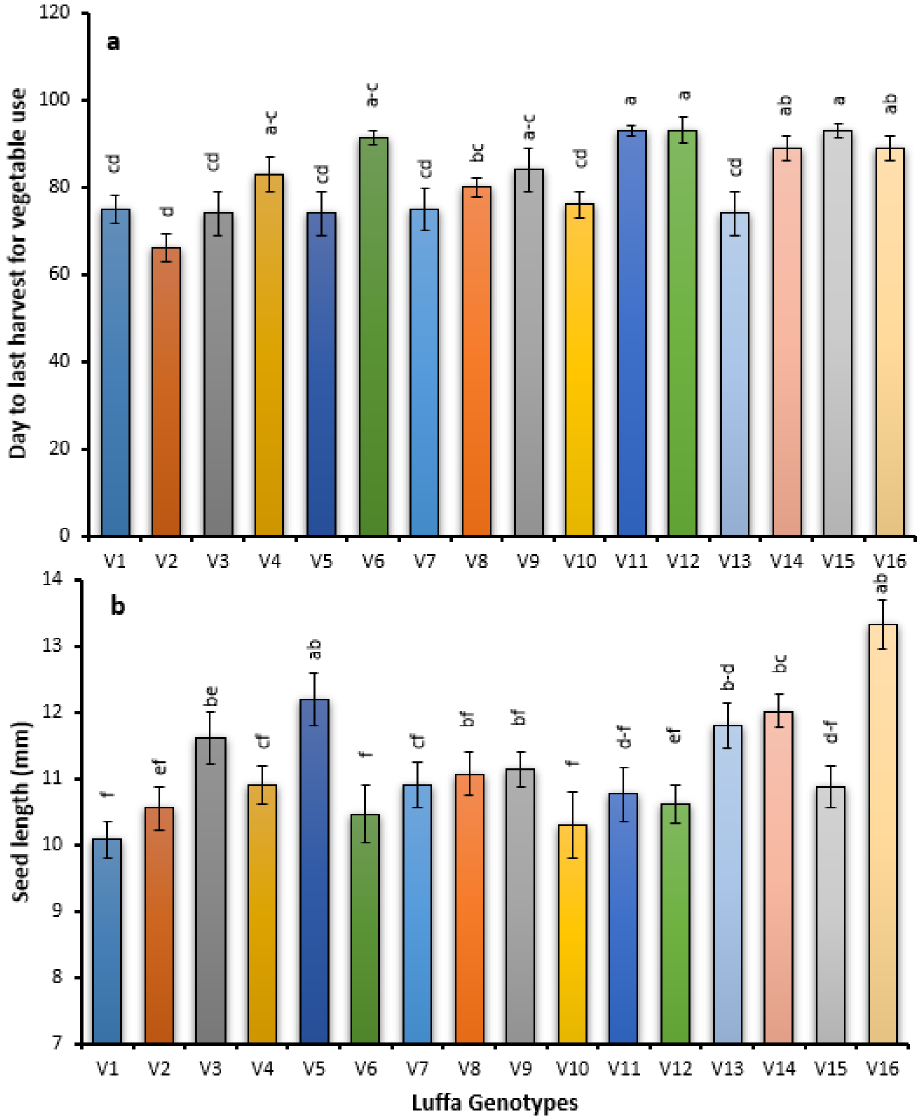


Fig. 5. Histogram for days to last harvest for vegetable use (a) and seed length (b) of various luffa genotypes. Error bars with standard deviation show relevant letters. Mean values with similar letters are statistically non-significant (P≤0.05).

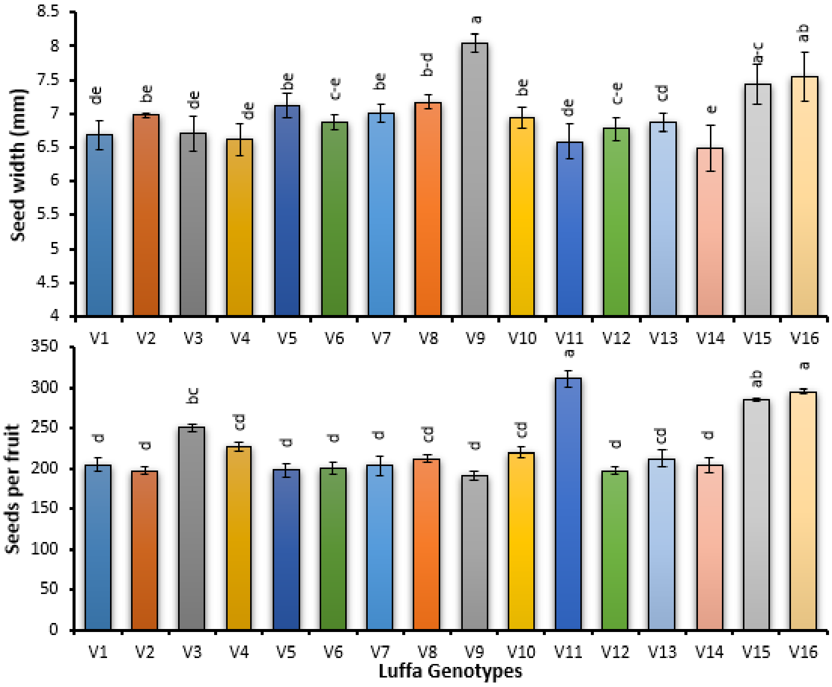


Fig. 6. Histogram for seed width (a) and number of seeds per fruit (b) in various luffa genotypes. Error bars with standard deviation show relevant letters. Mean values with similar letters are statistically non-significant (P≤0.05).

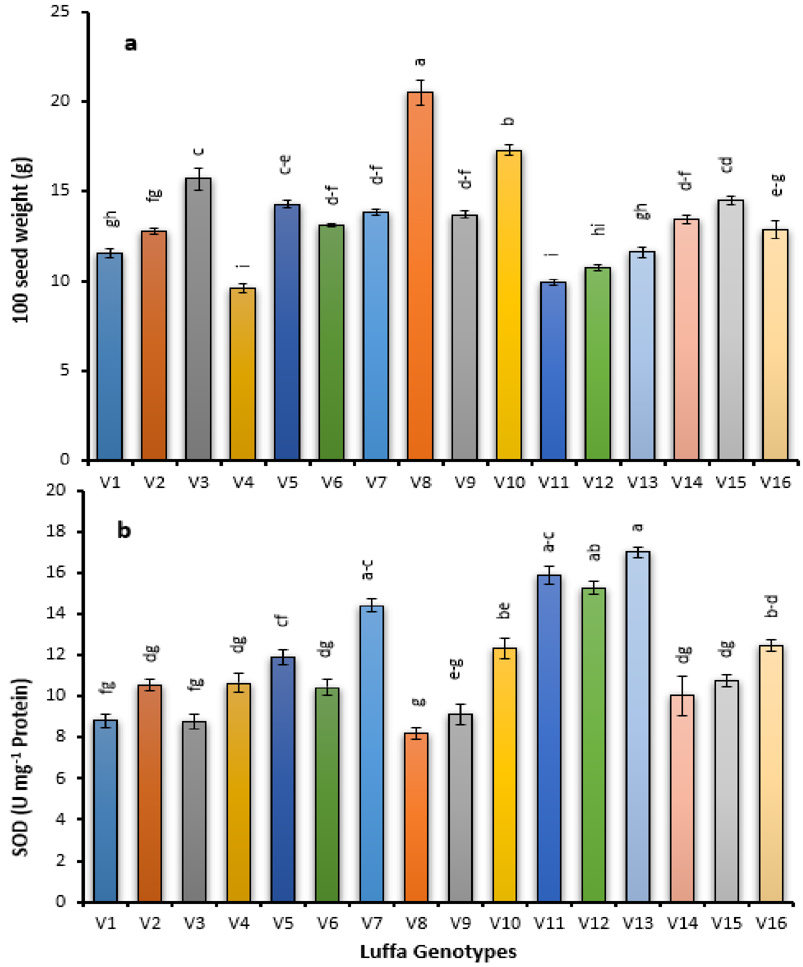


Fig. 7. Histogram for 100-seed weight (a) and SOD activity (b) in various luffa genotypes. Error bars with standard deviation show relevant letters. Mean values with similar letters are statistically non-significant (P≤0.05).

Under natural growth conditions, plants withstand many biotic and abiotic stresses, with an ultimate impact on growth and productivity. However, these stresses affect plants via various enzymatic activities, e.g., superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and protein contents (mg g^{-1}). Superoxidase dismutase (SOD) activity was affected by genotype variation (Fig. 7b). Maximum SOD activity appeared in V_{13}, followed by V_{11}, V_{12}, and V_7. A different response was found regarding peroxidase (POD) activity in luffa gourd genotypes (Fig. 8a). V_2 marked the highest activity peak, followed by V_4 and V_8. A similar trend occurred in CAT activity, where V_8 had superiority, followed by V_2, and the least activity was found in V_{16} (Fig. 8b). Protein contents are one of the important aspects of crop quality. Maximum protein contents appeared in V_{16}, while the minimum value occurred in V_9 (Fig. 9a). Chlorophyll content is an important component of crop health and productivity. Maximum chlorophyll contents were found in V_3 (30.67), followed by V_{11}, V_8, and V_5, while the minimum chlorophyll content (14.51) was found in V_{14} (Fig. 9b). The concentrations of nitrogen (N), phosphorus (P), and potassium (K) appeared in various luffa gourd genotypes (Fig. 10a, 10b, and 11a, respectively). The highest N contents were recorded in V_3 and V4 with a similar value of 2.06% (Fig. 10a). Regarding phosphorous, V_2 and V_8 shared a value of 0.08% (Fig. 10b), and regarding potassium, V_8 showed the highest value (12%) among all genotypes (Fig. 11a).

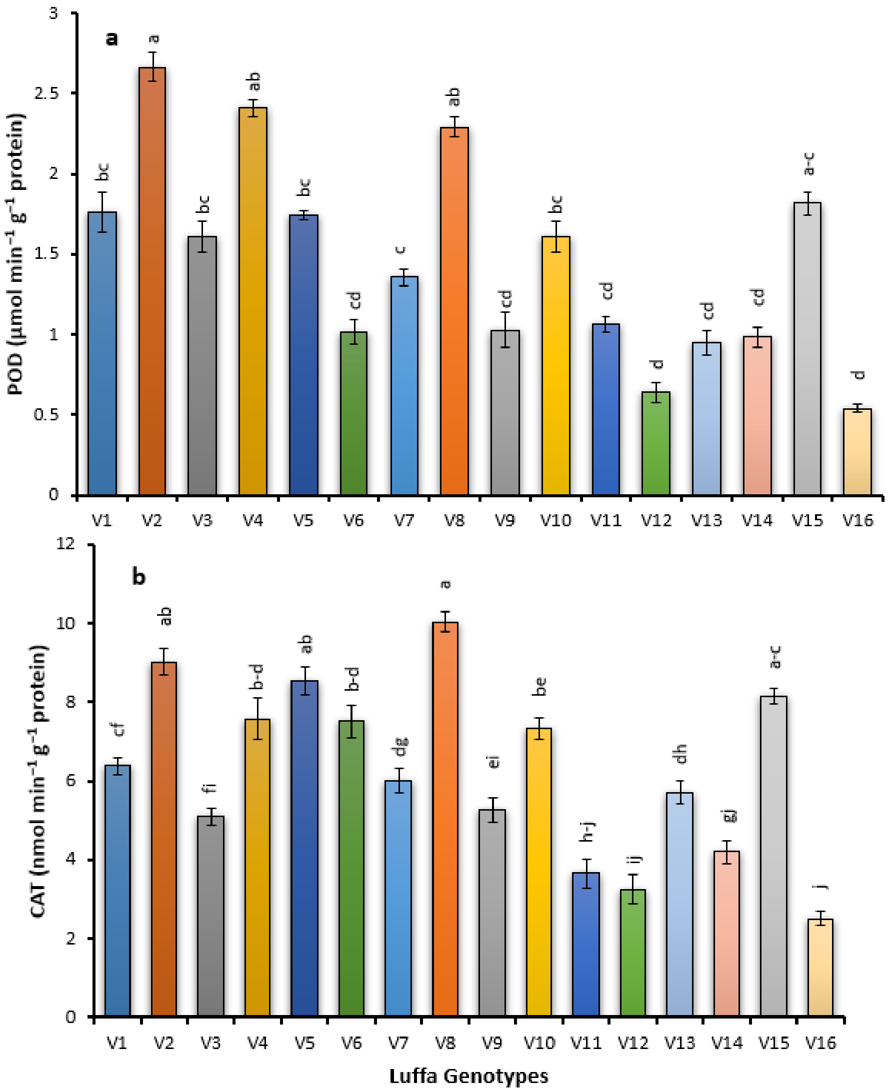


Fig. 8. Histogram for POD (a) and CAT activity (b) in various luffa genotypes. Error bars with standard deviation show relevant letters. Mean values with similar letters are statistically non-significant (P≤0.05).

Principal component analysis

PC analysis showed 78.99% total variation among the genotypes regarding vegetative, reproductive growth, fruit physical and biochemical attributes. Also, 42.05% of the total variation referred to mineral composition, reproductive features, and biochemical traits (Fig. 12a), thus demonstrating 24.88% by factor 1 and 17.17% by factor 2. An interesting scenario was that N contents positively correlated with chlorophyll content, followed by seed count and seed length. It also depicted that fruits enriched with K have higher fruit and seed weight, accompanied by high levels of POD and CAT. Fruit diameter positively correlated with fruit and seed width. Protein and SOD contents positively correlated and affected fruit and petiole length (Fig. 12a). According to the analysis, the second principal component (PCA) described 28.80% of the total variation (17.17% factor 2 and 11.63 factor 3). Similar to PCA-1, N contents positively correlated with chlorophyll, POD, and CAT, whereas fruit weight positively correlated with P and K. However, these two mineral elements correlated negatively with SOD contents. The number of seeds correlated positively with seed length and 100-seed weight. Regarding biochemical profiling, CAT and POD correlated closely and antagonistically with SOD and protein content (Fig. 12b).

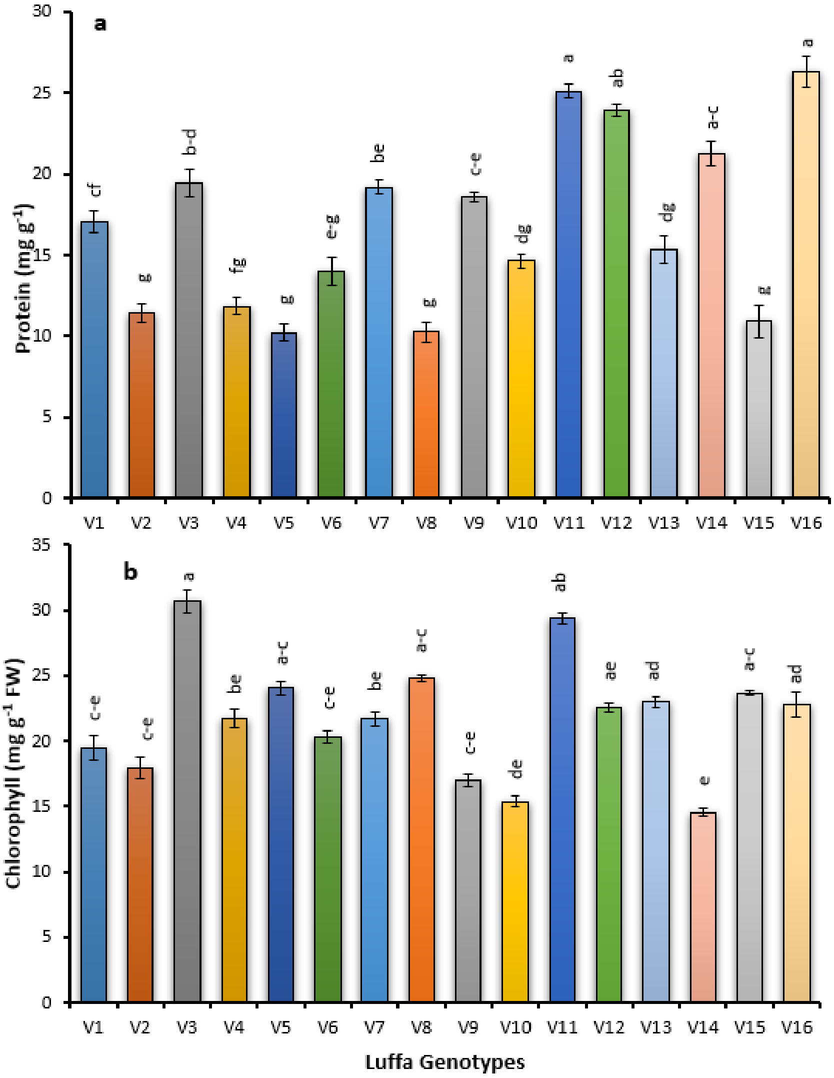


Fig. 9. Histogram for total protein (a) and chlorophyll contents (b) in various luffa genotypes. Error bars with standard deviation show relevant letters. Mean values with similar letters are statistically non-significant (P≤0.05).

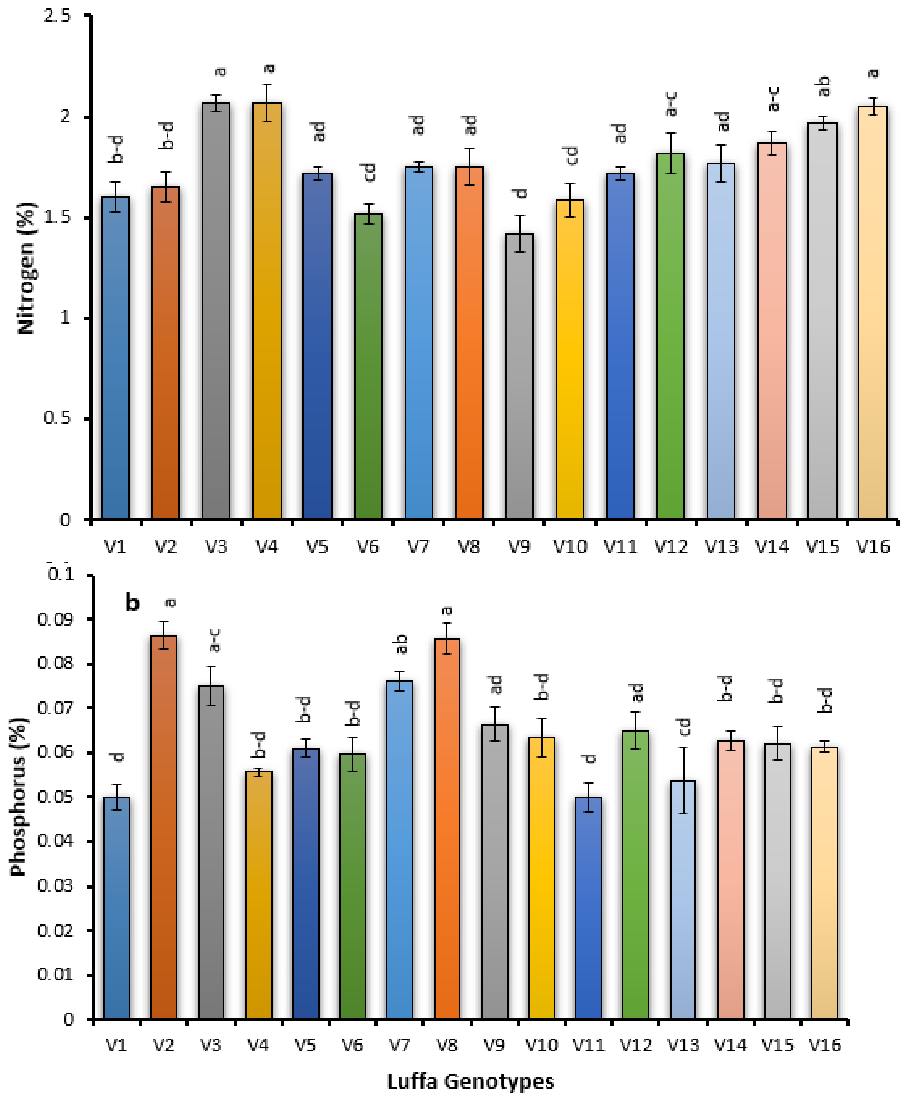


Fig. 10. Histogram for nitrogen (a) and phosphorus contents (b) in various luffa genotypes. Error bars with standard deviation show relevant letters. Mean values with similar letters are statistically non-significant (P≤0.05).

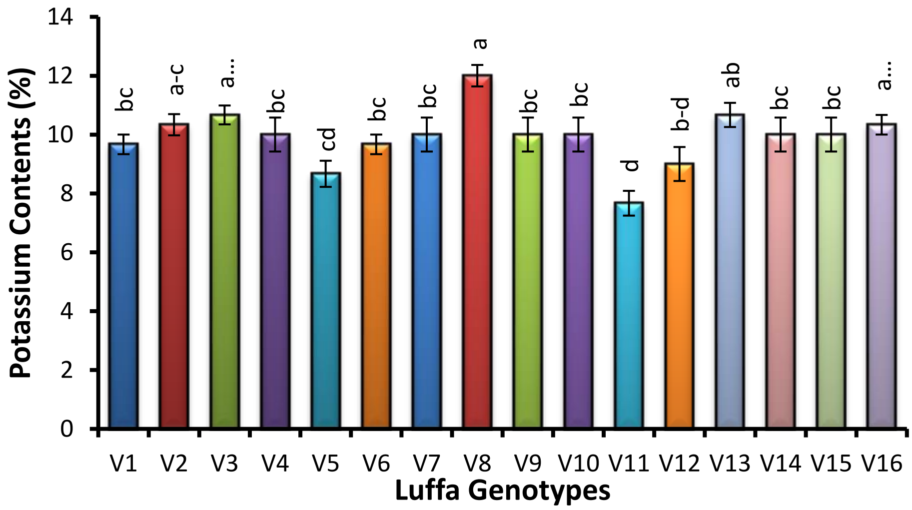


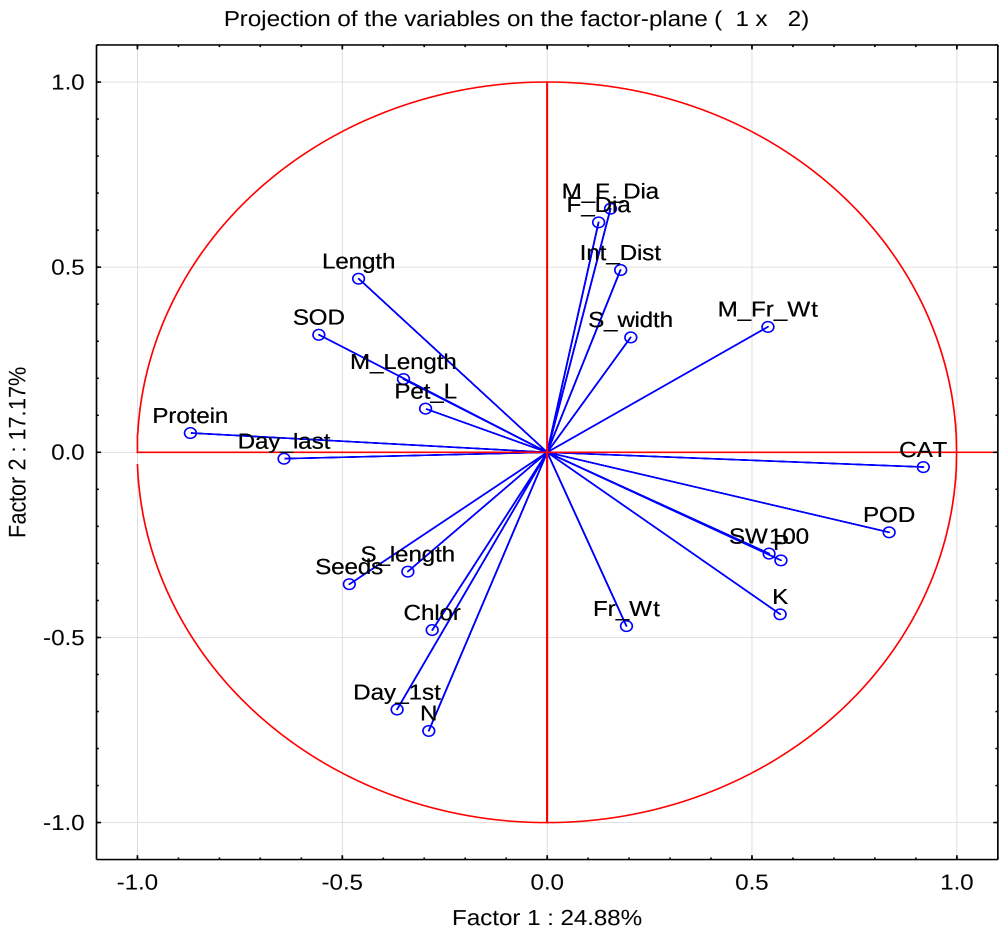
Fig. 11. Histogram for potassium contents in various luffa genotypes. Error bars with standard deviation show relevant letters. Mean values with similar letters are statistically non-significant (P≤0.05).

Cluster analysis

All sixteen luffa gourd cultivars were categorized into two main groups. Group 1 contained 9 luffa cultivars (All green, Advanta 1103, Nutech India, HDH 245, Sponge gourd AVANTI, Jaipuri Long, Sponge gourd hybrid, Dilpasand and Ridge gourd 619). These nine cultivars were subdivided into 2 subgroups. Subgroup 1 exhibited an average fruit diameter, fruit fresh weight, number of seeds per fruit, and seed width. Subgroup 2 depicted that HDH 245 and Advanta 1103 had significant seed width, petiole length, and fresh fruit length, while Jaipuri Long, Ridge gourd 619, Sponge gourd hybrid, and Dilpasand recorded higher fresh and mature fruit length and weight, phosphorus and potassium contents, seed count per fruit and 100-seed weight. Group 2 included seven luffa cultivars (Sponge gourd 1, 2, and 3, Sponge gourd Local and GARIMA, Ridge gourd 625 and Gloob F_1 hybrid) which further separated into 3 subgroups. Subgroup 1 had 4 genotypes, subgroup 2 had 2 genotypes, and subgroup 3 had 1 genotype. Group 2 was categorized based on petiole length, mature fruit diameter, days taken to first harvest, seed length, and N% (Fig. 13).

Correlation matrix

Correlation analysis of traits in all varieties showed that yield had the highest positive correlation (0.843) with fruit weight, followed by the second highest positive correlation (0.802) with fruit count. Among other qualitative traits, a high correlation (0.830) occurred found among peroxidase (POD) and catalase (CAT) contents (mg g^{-1}), and fruit count also correlated positively (0.675) with fruit length. However, correlation analysis showed a significant association of 100-seed weight with phosphorus (0.629) and potassium (0.626), respectively. Another positive correlation (0.500) was observed between seed length and nitrogen content, thus proving the crucial value of these nutritional elements for seed growth and health. However, a highly negative correlation (-0.964) and (-0.776) was observed among proteins with POD and SOD contents, respectively. In general, plant yield correlated positively with fruit length, width, phosphorus and potassium contents, fruit and petiole length, number of seeds, and CAT content, while correlating negatively with nitrogen levels, SOD and protein contents, internodal distance, and days to first harvest (Table 4).



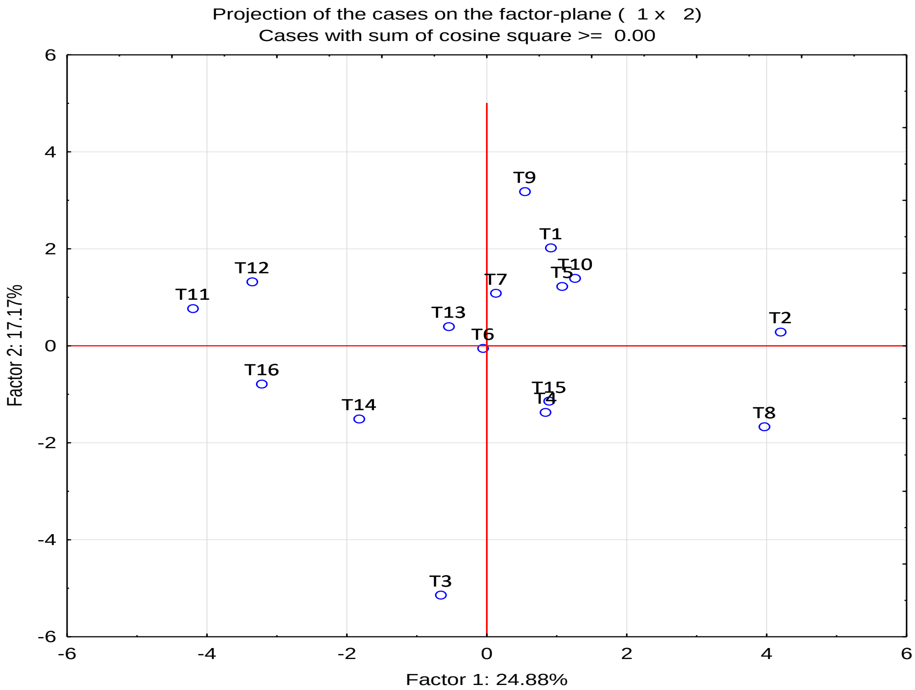


Fig. 12. Plot of principal components (a) and scatter plot (b).

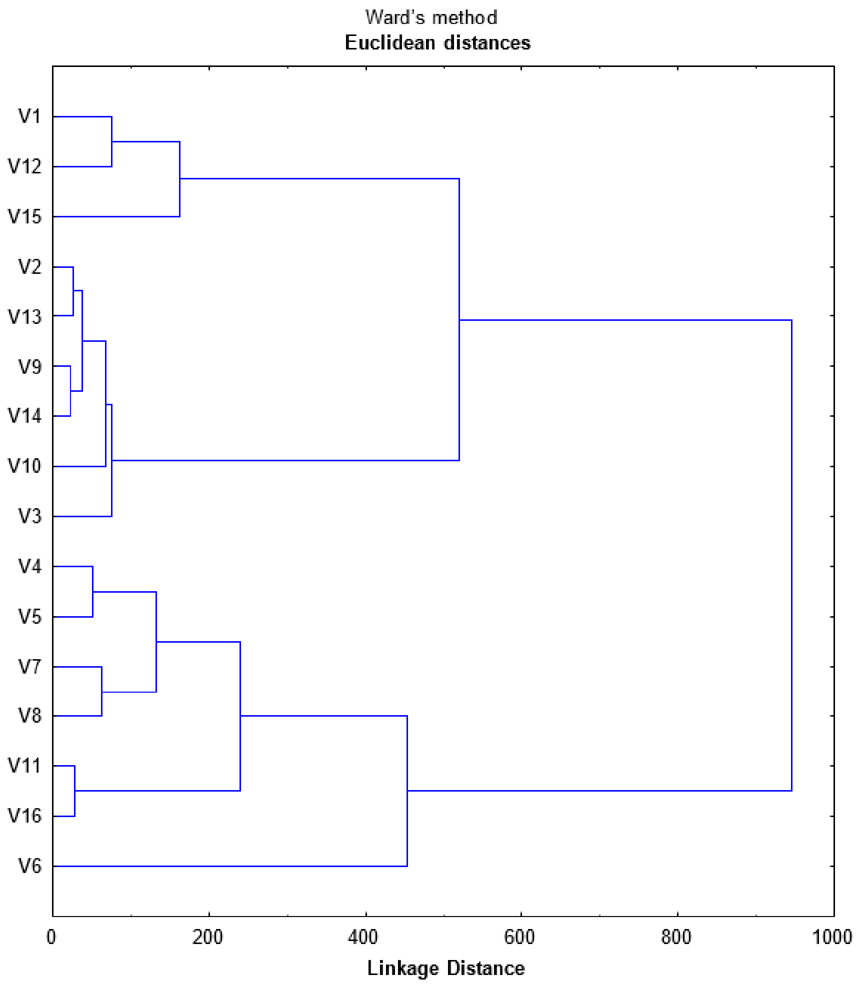


Fig. 13. Cluster analysis of luffa genotypes.

Discussion

Improvements in available germplasms of neglected and minor vegetable crops require characterization and conservation studies. The present study depicts substantial variability among morphological and biochemical characters in the luffa germplasm available throughout the country. Morphological characteristics among luffa gourd species were reported earlier by Joshi et al. (2004). Since all genotypes were evaluated with particular emphasis on diagnostic features (Tables 2 and 3), luffa gourds were measurable in growth habit, tendrils, leaf shape, leaf margin, leaf lobes, flower color, fruit color and ribs, seed color, stem, and blossom end fruit shape. These were similar to earlier reports by Joshi et al. (2004). However, they differed from each other regarding growth habits that ranged from intermediate to bushy with a ratio (11: 5) among genotypes. It was the case for leaf shape that varied from reniform to orbicular, while dented leaf margins were prominent in comparison to smooth margins. Thus, varied leaf patterns may be ascribed to diverse combinations of vascular patterns, leading to different physiological activities (Niinemets et al., 2007).

Regarding phenotype in luffa gourd tendrils, flower color, fruit ribs, fruit, and seed color, less genetic variation occurred among the genotypes. Low potential attributes in comparison to other traits for assessing genetic variability occurred, whereas stable features that were not affected by the environment made them useful for species delineation (Ajuru and Okoli, 2013), thus helping in genotype categorization regardless of geographic variability. It shows an insignificant relationship between geographic divergence and genetic diversity (Choudhary et al., 2011). Marr et al. (2005) stated that luffa had yellow-colored flowers. A similar pattern was followed in the case of fruit color that varied from light green to green. So was the pattern in seeds that had black seed color in abundance (Table 2). Determining variation in yield and its components of different luffa gourd genotypes will determine to what extent an environment affected yield (Ahmed and Khaliq, 2007; Ullah et al., 2012). On the contrary, it was also observed that individual yield components are minutely affected by the environment, as compared to yield itself. Thus, an assortment of such yield components (fruit length, width, weight, and fruit count per plant) served as prominent traits (Gatti et al., 2005) for ensuring good yield with enhanced quality (Maryam et al., 2015). Similar results were reported in cucurbits by Kumar et al. (2013).

Cucurbitaceae is a major plant family, rich in protein contents and antioxidative enzyme activity (Yasir et al., 2016). In the current research, rich biochemical diversity was assessed among luffa cultivars. However, GARIMA, AVANTI, and HDH-245 showed more SOD activity as compared to others under observation (Fig. 7b). Superoxide dismutase (SOD) is one of the major antioxidant enzymes involved in catalyzing the dismutation of O_2 to H_2O_2 and O_2. Therefore, this plays an imperative role against several environmental stresses by detoxifying reactive oxygen species (ROS) generated under stress conditions (Alscher et al., 2002). While our results expressed suitable levels of SOD in luffa fruit ranging from 8.20 U g^{-1} FW to 17.01 U g^{-1} FW (Fig. 7b). Similar results have been observed by Wang et al. (2010). Peroxidase (POD) is another valuable secondary enzyme that catalyzes oxidoreduction among phenolic substrates and H_2O_2. Our results expressed ample levels of POD particularly in ADVANTA-1103, Sponge gourd-Local, and Sponge gourd-3. Healthy plant growth and yield attributes in these accessions could be attributed to the profound activities of these enzymes in major physiological and regulatory reactions, i.e., MDA production, regulation of senescence, and defense behavior under normal and stress conditions. Similar variations among POD levels occurred in fruits of *L. aegyptiaca* (Yadav et al., 2011).

Catalase (CAT) enzyme also depicted significant variations among fruits of variant luffa gourd cultivars (Fig. 8b). Catalase also plays an important role in the conversion of H_2O_2 into H_2O_2 and O_2 (Sofo et al., 2015). Luffa gourd cultivars Sponge gourd-3, Dilpasand, and Ridge gourd 625 exhibited higher levels of catalase enzyme. Similar variations in catalase contents of luffa cultivars were reported by Singh et al. (2010). These results also suggest that CAT activity is positively correlated with yield and growth attributes of luffa gourd, thus enhancing these characteristics via strengthening the plant defense against biotic and abiotic constraints (Silva et al., 2018). Four accessions of the luffa gourd (Gloob F1-Hybrid, Garima, Avanti, and Jaipuri long) exhibit high protein levels (21.24-26.29 mg g^{-1} fresh weight). These findings confirmed conclusions by Amoo et al. (2008) with a similar generation of protein levels.

Table 4. Correlation matrix of luffa gourd genotypes, showing their reproductive, nutritional, and biochemical traits.

	Fr_Wt	M_Fr_Wt	F_Dia	M_F_Dia	Length	M_Length	Int_Dist	Pet_L	Chlor	N	P	K	SOD	POD	CAT	Protein	S_width	S_length	Day_1st	Day_last	Seeds	SW100	St_dia	Fruits	Yield
Fr_Wt	1.000																								
M_Fr_Wt	0.026	1.000																							
F_Dia	-0.277	0.464	1.000																						
M_F_Dia	-0.382	0.013	0.617	1.000																					
Length	-0.107	0.011	0.128	0.263	1.000																				
M_Length	0.066	0.174	0.031	-0.212	0.447	1.000																			
Int_Dist	0.010	0.026	0.202	0.358	0.148	0.068	1.000																		
Pet_L	-0.140	-0.059	-0.163	-0.275	0.230	0.014	-0.138	1.000																	
Chlor	0.208	-0.228	-0.177	-0.373	-0.278	0.060	-0.050	0.265	1.000																
N	0.192	-0.511	-0.227	-0.280	-0.355	-0.132	-0.376	-0.381	0.468	1.000															
P	0.304	0.365	0.047	-0.100	-0.045	-0.120	-0.184	-0.119	-0.019	-0.002	1.000														
K	0.288	0.240	-0.044	-0.042	-0.317	-0.210	-0.490	-0.305	-0.134	0.178	0.590	1.000													
SOD	-0.164	-0.212	0.160	0.252	0.299	0.364	0.072	-0.072	0.206	0.046	-0.357	-0.463	1.000												
POD	0.195	0.323	0.012	0.083	-0.621	-0.543	0.145	-0.319	-0.005	0.048	0.434	0.329	-0.493	1.000											
CAT	0.253	0.491	-0.023	-0.014	-0.528	-0.165	0.274	-0.272	-0.115	-0.240	0.405	0.375	-0.439	0.830	1.000										
Protein	-0.228	-0.333	0.060	-0.012	0.625	0.173	-0.308	0.359	0.143	0.167	-0.271	-0.363	0.394	-0.776	-0.964	1.000									
S_width	-0.060	0.483	0.518	0.112	0.125	0.316	0.172	-0.118	-0.146	-0.230	0.234	0.246	-0.199	-0.118	0.074	-0.084	1.000								
S_length	0.375	-0.299	-0.008	-0.070	0.203	0.301	0.096	-0.460	0.185	0.500	-0.027	0.148	0.093	-0.382	-0.380	0.300	0.275	1.000							
Day_1st	0.149	-0.538	-0.446	-0.372	0.050	-0.088	-0.377	0.041	0.259	0.584	0.204	0.128	-0.140	-0.199	-0.449	0.449	-0.359	0.437	1.000						
Day_last	-0.158	-0.135	-0.144	-0.428	0.232	0.544	-0.327	0.247	0.097	0.173	-0.414	-0.361	0.150	-0.557	-0.463	0.444	0.063	0.076	0.009	1.000					
Seeds	-0.009	0.013	-0.098	-0.435	-0.106	0.144	-0.233	-0.036	0.571	0.527	-0.295	-0.225	0.216	-0.176	-0.359	0.381	0.038	0.288	0.166	0.451	1.000				
SW100	0.102	0.420	-0.226	-0.214	-0.002	0.185	-0.087	-0.096	-0.026	-0.109	0.629	0.626	-0.473	0.286	0.492	-0.391	0.313	0.047	0.109	-0.260	-0.137	1.000			
St_dia	0.004	0.309	-0.303	0.022	0.173	0.125	-0.242	-0.061	-0.434	-0.465	0.192	0.351	-0.135	0.028	0.175	-0.131	-0.032	-0.179	-0.070	-0.084	-0.277	0.432	1.000		
Fruits	0.369	0.402	-0.161	-0.377	0.257	0.675	-0.072	0.368	0.096	-0.350	0.130	-0.039	0.079	-0.123	0.204	-0.102	0.117	-0.202	-0.213	0.282	-0.007	0.311	0.260	1.000	
Yield	0.843	0.247	-0.248	-0.431	0.093	0.443	-0.020	0.105	0.148	-0.094	0.215	0.153	-0.055	0.037	0.282	-0.214	0.022	0.126	-0.072	0.088	-0.031	0.215	0.146	0.802	1.000

In the present study, cluster analysis of 16 genotypes of luffa gourd was undertaken based on quantitative traits, which showed genetic diversity (Fig. 12a, b). Cluster analysis of luffa gourd previously clustered separately morphological similarities and differences, taxonomic variability, soluble proteins, and flower morphology (Singh and Bhandari, 1963; Chakravarty, 1982; Cruz et al., 1997; Pasha and Sen, 1991; Marr et al., 2005b). Similar results were also reported in ridge gourd (Gautam 2013), muskmelon (Trimech et al., 2013), and bottle gourd (Mladenovic et al., 2012).

Conclusion

In the present study, the luffa gourd accessions collected from all over Pakistan were characterized regarding morphological, reproductive, nutritional, and biochemical traits. The outcomes showed significant variations and diversity among the accessions. The results concluded 17.7% variation among the traits and 21.1% among the accessions. Among the accessions, the cultivars Sponge gourd 1102, Ridge gourd 625, and Gloob F1 Hybrid were the most favorable regarding premium morphological and qualitative traits. Thus, they could be used for further varietal improvement programs.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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