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ASSESSMENT OF MORPHOLOGICAL DIVERSITY AMONG INDIGENOUS CUCUMBER GERMPLASM OF PAKISTAN

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Cucumber, (*Cucumis sativus* L.) is an essential vegetable crop globally. A field study was conducted to assess morphological diversity of cucumber germplasm at Vegetable Research Area, Institute of Horticultural Sciences, University of Agriculture Faisalabad (UAF), Pakistan during the year of 2018 and 2019. Present study was designed with 25 genotypes of cucumber taken from National Agricultural Research Centre (NARC), Islamabad, Pakistan for determining their performance based on quantitative and qualitative characters. Cluster analysis and principle component analysis (PCA) were carried out for classifying cucumber genotypes on the basis of resemblances in the phenotypical attributes of fruits, vine, leaves, and flowers by employing XLSTAT (2018). Phenotypic diversity was highest in color of leaves, size of leaves, skin color of fruits and shape of fruits. Genotypes C-32149, C-574, Wandri 700, C-571 were closely associated to one another for several morphological attributes. Results of PCA analysis for the quantitative phenotypical attributes showed that major first two principle components (PCs) described 23.64% and 17.89% (Combined 41.53%) diversity. Cluster analysis of all cucumber genotypes of Pakistan classified them into main two clusters with 24.44% diversity within classes and 75.56% variability between the classes. Genotypes C-32149, C-574, Wandri 700 and C-571 showed greatest diversity various phenotypic traits including color of leaf, skin color of fruit and size of leaf.

Keywords: Cucumber, *Cucumis sativus*, morphological characterization, Genotypes assessment.

INTRODUCTION

Cucurbitaceae is the most genetically dissimilar group of plants in species kingdom Plantae. Genus cucumis has 32 species but more popular and widely cultivated is melon and cucumber (Stojilovic, 2007). Cucumber (*Cucumis sativus* L.) belongs to family cucurbitaceae which consists of about 960 species (Jeffery, 2005; Sachaefer *et al.,* 2009). It has been cultivated for atleast 3000 years (Ullah *et al.,* 2012). It is fourth important vegetable crop after cabbage, tomato and onion. As vegetable crop, it has great economic importance (Plader *et al.,* 2007). It is cultivated around the globe and is important member of cucurbitaceous crops (Jeffrey, 1980; Kirkbride, 1993; Li *et al.,* 2008). Cucumis genus is considered to be originated in Asia (Sachaefer *et al.,* 2009). Cucumber is generally eaten as raw fresh or in pickled form and it is important diet of the people living in south east Asia and Russia. Fruit of cucumber contains 90% water when in mature form, its texture and flavors make it important to use as salad as well as in pickles (Thompson and Kelly, 1959). In some countries, cucumber is used in curries and chutney preparation and also used in beauty aids as skin tonics (Whitaker and Davis, 1962). Cucumber is excellent crop for diabetic patients because it contains low sugar and aid to burn excess fat in the body (Wehner and Gunner, 2004). There are several kinds of cucumber fruits that are different in shape, color and size (Esteras, 2008). Fruit shape is significant quality factor for several domesticated plants (Grandillo *et al.,* 1996) and existing cultivars which are divided into many cultivar groups based on shape of fruit. Now, choice of consumer is determined by a series of individual preferences like shape, quality and size. According to the Food and Agriculture Organization of United Nation, 20% of cucumber crop was sold in fresh form (van Eck *et al.,* 1998). It is an important fruit due to more medicinal, nutritional and economic potential. With latest campaigns to encourage longevity and good health, it is predictable that consumption of vegetables, like cucumber can enhance in Pakistan which may leads to increased production of cucumber (Eneobong, 2001; Afangideh and Uyoh, 2007). Cucumber crop duration is shorter than most of other crops (Wehner and Guner, 2004). Morphological characterization is first most important step to describe and classify the genetic resources (Smith and Smith, 1989) and genetic diversity in crop plants (Decker and Wilson, 1986; Escribano *et al.,* 1991; Cartea *et al.,* 2002 Balkaya and Ergun, 2008). Additionally, length, diameter and color of fruit have been employed as important economic attributes (Kennard and Havey, 1995; Ahmed *et al.,* 2004). Phenotypic variation in cucurbit crops has been based upon the fruit characteristics (Balkaya *et al.,* 2005; Ferriol and Pico, 2008), which is useful for differentiation of interrelated species (Gwanama *et al.,* 2000).

Genetic diversity of plants is key constituent of every agricultural production system. This similarity or genetic diversity can be evaluated through many genetic markers. Theses have been employed to evaluate evolutionary relationship between and within species and genera (Paterson *et al.,* 1991). Genetic diversity analysis and relatedness within and between different populations, species and individuals is requirement towards plant genetic resources protection and efficient utilization (Weising *et al.,* 1995). Success of breeding program mainly depends upon genetic variation that is available in population (Afangideh *et al.,* 2005; Subramanian and Subbaraman, 2010). The present study was undertaken to assess diversity among morphological attributes and indirect and direct contribution of these attributes towards yield of cucumber germplasm and is important step in assessing plant materials as genetic resources for breeding program.

MATERIALS AND METHODS

Experiment was performed with four replications in randomized complete block design from March to August 2018 which was repeated for validation next year during March to August 2019 at experimental site of Institute of Horticultural Sciences (IHS) UAF, Faisalabad, Pakistan for assessing variations among cucumber germplasm collected from NARC, Islamabad, Pakistan. Data presented is an average of two year's study. The experimental place is located at latitude of 31° 22' N and longitude of 72° 56' E. Mean temperature and rainfall of the experimental site during the study year is shown in Figure 1. Cucumber seeds were sown directly in the field during the month of March 2018 on upraised beds with the dimension of 10′×5′ and plants were grown at spaced 1.5′×1.5′. Five seeds of cucumber were sown in each hole and thinning practice was done to one cucumber seedling subsequently three weeks of sowing seeds. Regular irrigation was applied as per recommendations of (Baloch, 1994). Flowers, vines, fruits and leaves of cucumber were studied from matured plant. Chemical treatment of seeds was practiced with Imedacloprid at concentration of 2.5g per kg before sowing. Different macronutrients phosphorous, potassium in the form of DAP and Nitrogen was applied in form of the urea, phosphorous and potassium @ 85:60:150 kg ha^{-1}. One 3rd of nitrogen was applied before sowing. Second dosage of nitrogen was applied at fruit bud formation and third was after the first fruit set. Irrigations and manual hoeing was done regularly twice a week throughout crop season. Data was taken on 19 morphological (14 quantitative and 3 qualitative) attributes including: Leaf fresh weight, number of leaves, leaf dry weight, petiole length, internodal distance, leaf area index, vine length, vine diameter, fruit length, fruit width, number of flowers per vine, fruit fresh weight, number of fruits per vine, weight of dry fruit, shape of leaf, size of leaf, color of leaf and skin color of fruit. Observations were recorded on four randomly selected plants of each germplasm per replication. Digital vernier caliper was employed to record diameter, length and thickness. Digital balance was used to measure weight of fruits. A detail of morphological attributes considered is given below:

***Morphological qualitative traits*:** Leaf size (small, medium, large), shape of leaf (cordate, angular,), color of leaf (light green, intermediary green and dark green) were analyzed visually at greenery stage. Floral parameters were studied at flowering stage. Cucumber fruit shape (cylindrical, spherical) was also observed at fruit mature stage. Skin color of fruit (light green, intermediary green, dark green) was evaluated visually. Fruit characteristics were premeditated at full matured phase (Srivastava *et al.,* 2001).

***Morphological quantitative traits*:** Number of leaves was counted considering four plants from each repetition and was averaged. Leaf area index was calculated through randomly selected leaves from each of the replication, removed sampled leaves from plants and placed upon leaf area meter (LI-3100; Inc., Lincoln, Nebr) and averaged to compute leaf area in centimeters. A ratio between leaf area to the area of ground was used to calculate leaf area index. Vine length was measured by using ruler. Diameter of vine was recorded with help of the vernier caliper (KBD-MT 0014). Number of flowers was counted and recorded. Cucumber fruits were harvested from the field at ripening period by cutting fruits using nail clippers. Harvested fruits of cucumber were instantly taken to the vegetable lab. Width and length of fruit was measured with help of paquimeter (KBD-MT-0014). Fruits per vine were counted and noted. Dry and fresh weights of fruits were calculated by employing weighing balance Hytek (SF-400 A). Weight of fresh fruit was instantly taken in the field and then placed at room temperature indoor for loss of moisture then after two day's cucumber fruits were placed in at 72°C in microwave oven till constant dry weight was attained.

***Statistical analysis*:** Data taken were statistically evaluated employing computer software (XLSTAT 2018). Analysis of variance (one way) was used to evaluate similarities and dissimilarities among cucumber germplasm. A significant variation between means was noted by Duncan multiple range (DMR) test at $P<5\%$ in data of quantitative morphological attributes. Multivariate relationship among germplasm was recorded through principle component analysis on basis of resemblances in morphological attributes. Images of the morphological attributes of each germplasm employed for experiment were also taken using an android phone Honor 3C. Multivariate analysis of variance analysis, cluster analysis and principle component analysis were performed for assemblage cucumber germplasm on basis of resemblances in quantitative morphological attributes of vine, leaves, fruits and flowers by employing XLSTAT (2018). Analysis of variance was performed for significant dissimilarities between means were noted by DMR at $P<5\%$ in quantitative

morphological attributes. Statistical analysis was performed on 19 morphological characters (14 quantitative, 3 qualitative) discoursed above in the detail. Descriptive statistics (standard deviation, coefficient of variation and mean) were analyzed employing multivariate analysis of variance analysis. Eigen values and scores were calculated.

RESULTS

For the morphological diversity, 25 cucumber genotypes were assessed for vine, leaf, fruits and flowers traits. Quantitative traits that were assessed includes weight of fresh leaf (gm), leaf area index, weight of dry leaf, number of leaves, vine length, vine diameter, number of flowers per vine, number of fruits per vine, intermodal distance, length of fruit, petiole length, weight of dry leaf, weight of fresh leaf and width of fruit. A great deal of diversity in morphological parameters (quantitative and qualitative) was observed among cucumber germplasm of Pakistan. Diversity in three qualitative traits of 25 cucumber germplasm is shown in Table 1. Morphological diversity was greatest evident in skin color of fruit, color of leaf and size of leaf. A qualitative character was categorized into many different classes for each of the variable tested. For instance, all three classes of color of leaf were analyzed as dark green, intermediary/medium green and light green. Leaf size was observed as small, medium and large in the assemblage.

Fruit shape varied from cylindrical to spherical. Skin color of fruit was also identified as dark green, intermediary/medium green and light green. An overall view indicating some of observed diversity in size of leaf, color and shape is shown in Figure 1. Likewise, Figure 2 shows morphological variation in fruit color, shape and size. All quantitative characters varied significantly. For instance, number of leaves varied from 92 to 502, whereas, weight of fresh and dry leaf (gm) varied from 7.32 (gm) to 1.33 (gm) and 2.19 (gm) to 0.23 (gm) correspondingly.

Results of principle component analysis (PCA) for the quantitative phenotypic attributes showed that major first two principle components described 23.64% and 17.89% (total of 41.53%) of the total diversity (Table 3). PCA for qualitative phenotypic traits showed that major first two principle components described 41.81% and 39.01% (total of 79.82% of total diversity (Table 5). Leaves fresh weight, weight of dry leaves, vine length, number of fruits per vine contributed to PC1, while the number of leaves, leaf area index, number of flowers per vine, weight of fresh and dry fruit had largest contribution to PC2. The two dimensional plot of all genotypes according to their diversity is shown in Figure 3.

Table 1. Qualitative phenotypical variation in 25 cucumber germplasm of Pakistan.

Germplasm	Size of leaf	Color of leaf (green)	Skin color of fruit
Pico- 500	Small	Intermediary green	Light green
Wandri- 700	Large	Light green	Dark green
Cucumber Vega	Small	Light green	Dark green
C-574	Medium	Light green	Dark green
C-571	Large	Light green	Dark green
C-3479	Medium	Light green	Light green
C-3426	Medium	Intermediary green	Light green
C-3429	Small	Light green	Light green
Sialkot Local	Small	Light green	Dark green
KN-84604105	Small	Light green	Dark green
Desi Cucumber	Medium	Intermediary green	Intermediary green
C-3428	Large	Light green	Intermediary green
C-28295	Large	Light green	Light green
C-28522	Small	Intermediary green	Dark green
C-29643	Medium	Light green	Light green
C-32027	Small	Light green	Dark green
C-32031	Small	Light green	Dark green
C-32149	Medium	Intermediary green	Dark green
C-32805	Small	Intermediary green	Dark green
C-35830	Small	Light green	Dark green
C-35831	Medium	Light green	Light green
C-36833	Medium	Light green	Light green
Cucumber Ana	Medium	Intermediary green	Light green
Long green	Small	Intermediary green	Light green
Cucumber long	Medium	Intermediary green	Light green

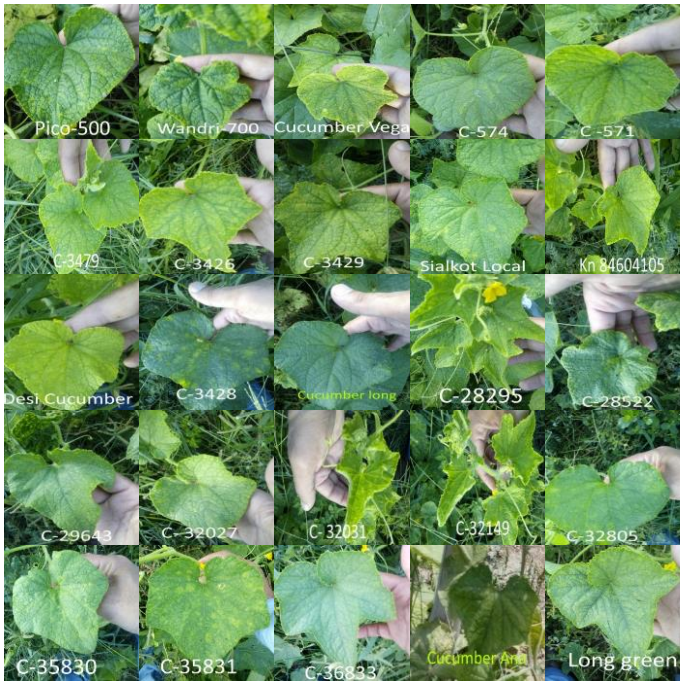

Figure 1. Pictures illustrating phenotypic variations in leaves size, shape and color of cucumber (*Cucumis Sativus*) obtained from NARC, Islamabad, Pakistan.

Figure 2. Pictures indicating phenotypic variations in fruit size, shape and skin color of cucumber genotypes obtained from NARC, Islamabad, Pakistan.

Results of cluster analysis employing data of morphology are shown in figure 4 and Figure 6. Cluster analysis of each cucumber genotype from Pakistan clustered accessions into main two clusters with 75.56% variability between classes and 24.44% diversity within classes which is demonstrated in Figure 4. Relatively, highest morphological variation was

seen between classes. Class I had genotypes Pico-500, C-3426, C-3429, Sialkot local, C-3428, C-28295, C-28522, Long green, C-28643, C-35831, C-35830, C-36833 which distinguished from the other classes. Class II had genotypes Wandri-700, Cucumber vega, C-574, C-571, C-3479, KN-84604105, Desi cucumber, C-32149 which showed diversity from Class III, Class I, Class IV and Class V. Class IV had genotype C-32805 which indicated lowest diversity between classes which showed in Figure 4.

Table 2. Descriptive statistics of phenotypical quantitative attributes of 25 cucumber germplasm of Pakistan

Variable	Min	Max	Mean	SD
Number of leaves	92.00	502.00	297.76	146.04
Fresh leaf weight	1.33	7.32	3.74	1.47
Dry leaf weight	0.23	2.19	1.01	0.65
Leaf area index	0.01	0.07	0.03	0.02
Vine length	108.00	210.00	153.76	24.26
Vine diameter	0.44	5.81	0.74	1.06
Number of flower per vine	95.00	516.00	323.00	134.32
Fruit length	7.00	25.00	13.80	4.00
Internodal distance	8.00	19.00	12.0	3.00
Width of fruit	3.20	6.40	4.87	0.89
Number of fruit per vine	1.00	5.00	2.96	1.17
Fruit fresh weight	35.00	740.00	191.28	182.60
Fruit dry weight	2.33	49.33	12.58	11.92
Length of petiole	5.00	21.00	13.28	4.43

Table 3. The major first two PCs (Principle components) of 14 quantitative morphological attributes.

Variable	PC1	PC2
Number of leaves	-0.40	0.06
Leaf fresh weight (gm)	0.46	0.00
Weight of dry leaf (gm)	0.40	-0.08
Leaf area index	-0.30	0.06
Vine length	0.20	-0.09
Vine diameter	-0.02	-0.08
Number of flower per vine	-0.11	0.06
Length of fruits	-0.41	-0.25
Internodal distance	-0.16	-0.19
Fruit width	-0.09	-0.34
Number of fruit per vine	0.14	-0.38
Weight of fresh fruit (gm)	-0.06	0.52
Weight of dry fruit (gm)	-0.06	0.52
Petiole length	-0.26	-0.20
Eigen value	3.30	2.50
Variability	23.63	17.88
Cumulative	23.63	41.52

DISCUSSION

Cucumber is an important vegetable crop worldwide. The scope of any crop depends on the genetic diversity magnitude present in available germplasm. Greater the diversity in available genotypes, greater would be chances of selecting superior germplasm (Simmonds, 1962).

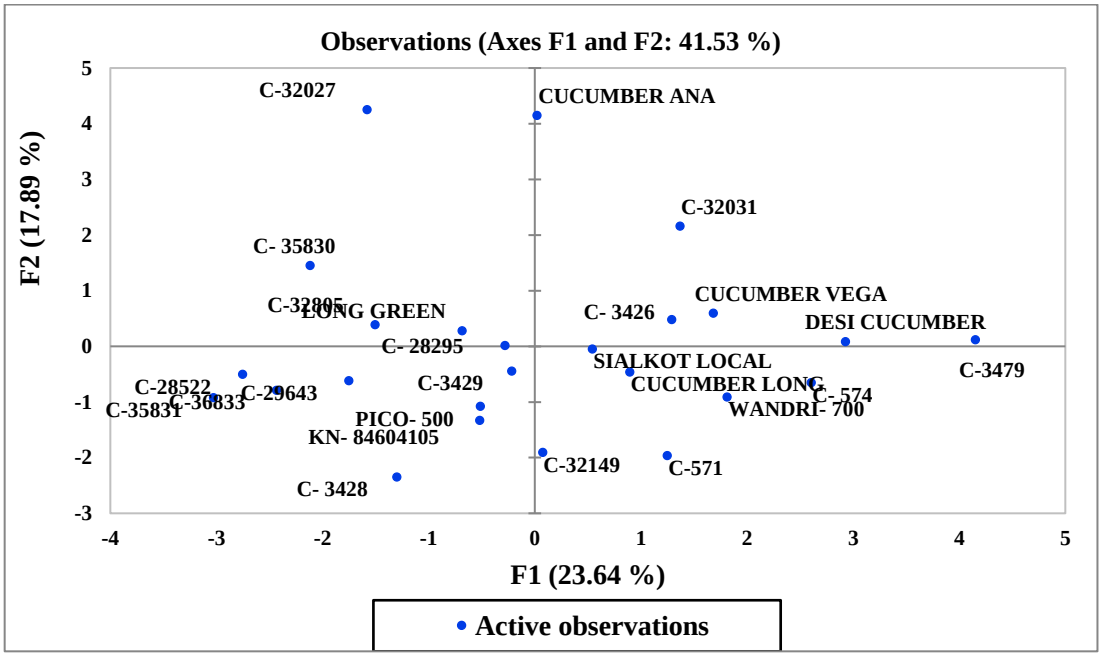

Figure 3. PCA plot of 25 cucumber germplasm based upon first major two components for morphological quantitative attributes.

Table 4. Descriptive statistics of phenotypical qualitative attributes of twenty-five cucumber germplasm of Pakistan.

Variable	Min	Max	Mean	SD
LSS	1.00	3.00	1.72	0.73
LC	3.00	5.00	3.72	0.98
FSC	3.00	7.00	5.08	1.95

Table 5. The major first two PCs (Principle components) of 14 qualitative morphological attributes.

Variable	PC1	PC2
LSS	-0.495	-0.656
LC	-0.350	0.751
FSC	0.795	-0.078
Eigen value	1.220	1.170
Variability	40.810	39.000
Cumulative	40.811	79.810

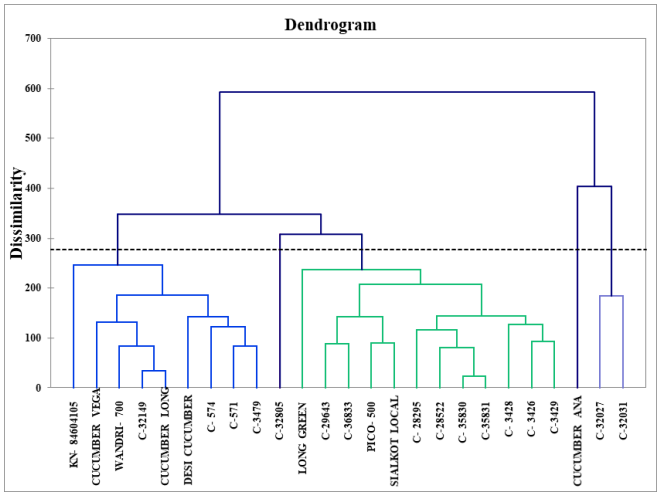

Figure 4. Cluster dendrogram of cucumber germplasm from Pakistan showing variation based upon phenotypical quantitative traits.

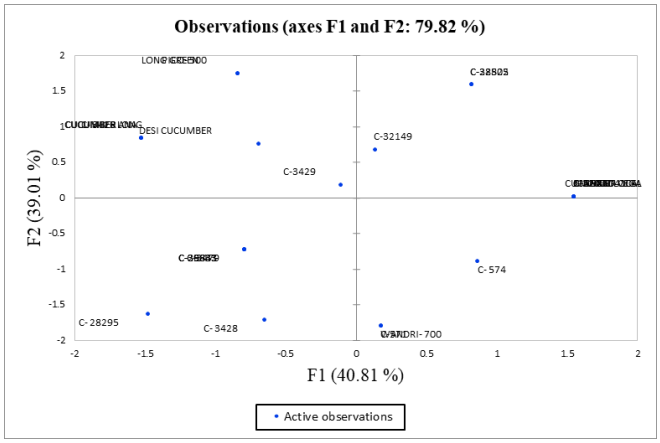

Figure 5. PCA plot of cucumber 25 germplasm based upon first major two components for morphological qualitative attributes.

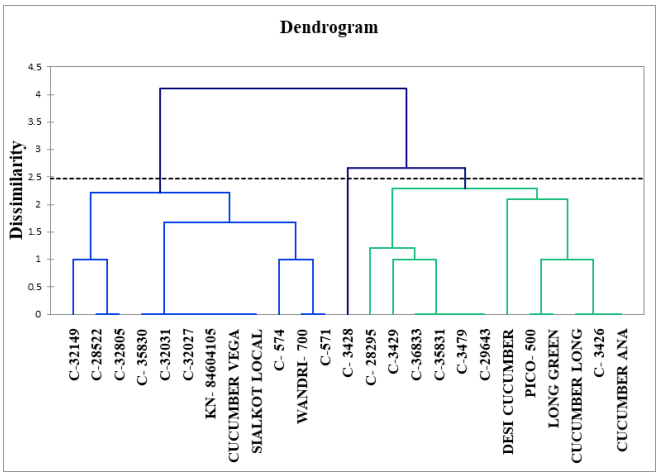

Figure 6. Cluster dendrogram of cucumber germplasm from Pakistan showing variation based upon phenotypical qualitative traits.

Cucumber genotypes of Pakistan acquired from National Agricultural Research Centre, Islamabad, Pakistan depicted greater morphological variation in the qualitative attributes which includes size of leaf, color of leaf and shape of leaf, skin color of fruit and fruit size in addition to quantitative traits including leaves fresh weight, number of leaves, leaves dry weight, length of vine (cm), diameter of vine (cm), number of fruits per vine, length of fruit, width of fruits, weight of dry fruit (gm) and weight of fresh fruit (gm). However, flower color and tendrils types were constant for all genotypes. These conclusions were congruous with findings of Abusaleha and Dutta (1990), Hossain *et al.* (2010) and Khan *et al.* (2015). Sharma *et al.* (2000) also observed significant diversity in length of cucumber fruits and diameter of fruits (Saha et al., 1992). Our results demonstrated immense deal of genetic variability in collected genotypes from Pakistan guiding towards possibility of the center of origin for this crop plant.

Coefficient of variability for phenotypic traits was observed high for length of vine accounted by Khan *et al.* (2015). Yield of fruit per plant had significant positive correlation coefficient with weight of fruits and number of fruits per plant reported by (Solanki and Seth, 1980). Weight of fruit had maximum positive involvement towards yield, followed by days to first flower appear and number of plants per vine. Attributes like fruit diameter, first female flower appearance and length of vine. Such characters are more under the effect of climatic conditions do not examined to selection. These conclusions were similar given by Choudhry and Mandal (1987).

Morphological features for instance leaf blade shape and petal color could not be adequate to demonstrate associations amid the species (Lloyd *et al.*, 1992). Genetic markers are employing to evaluate phylogenetic association and genetic variations in cucumber accessions. Different molecular

markers such as ISSR, EST-PCR, RAPD, SRAP and EST-SSR have been used for the evaluation of phylogenetic association and genetic variation of cucumber genotypes with the other cucurbit crops (Levi *et al.,* 2005, 2010). Few reports on the RAPD study in the available cucumber germplasm indicated genetic distance in different ranges between genotypes under study that concluded genetic variation among 118 cucumber genotypes (Stub *et al.,* 1997). Lowest genetic distances were observed between 0.01 and 0.58 which showed limited genetic variation in the available genotypes. Chen *et al.* (2006) evaluated genetic distance between genotypes which ranges from 0.064 to 0.59 with mean value of 0.24 that indicated wide genetic variation in genotypes. (Ahmed *et al.,* 2003) evaluated genetic distances among African genotypes which ranged in between 0.36 and 0.88.

Evaluation of genetic variation of our assemblage by the genetic markers would be valued to additional categorize molecular background of Pakistani cucumber. In this experiment seeds of characterized cucumber germplasm are conserved at 4˚C in Vegetable Stress Physiology Lab, Institute of Horticultural Sciences, (IHS), University of Agriculture, Faisalabad from where the sample seeds can be taken from corresponding author.

***Conclusion*:** The present experiment concluded that morphological diversity was greatest evident in color of leaf, skin color of fruit, size of leaf and fruit shape. Genotypes C-32149, C-574, Wandri 700, C-571 were closely associated to one another for several morphological attributes.

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