Research Article

Morphological and agronomic characterization of (*Capsicum annuum* L.) germplasm in Pakistan

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Abstract								

Abstract

The aim of the study was to estimate the magnitude of genetic variation through the characterization of germplasm using agronomical and morphological traits. In the current investigation 38 genotypes of chilli (*Capsicum annuum*) were evaluated at an agronomical and morphological level for (10 quantitative and 14 qualitative traits). A set of parameters covering different growth stages selected from the *Capsicum* descriptors developed by Biodiversity International Former. Qualitative traits were studied on a visual basis. Quantitative characters such as plant height ranged from 40 cm (36588) to 63 cm (36616). Fruit length and width range from 2.21 cm (36591) to 13.2 cm (36636) and 0.60 cm (36569) to 2.90 cm (36560 and 36441). Fruit weight varied from 0.85 g (36591) to 8.30 g (36651). Results obtained for yield/plant ranged from 175.49g (36652) to 780.3 g (36616). Results demonstrated the existence of diversity between the studied genotypes and consequently, providing the opportunity of attaining genetic advances in hybridization program for particular traits under consideration. **Keywords**: Agronomic traits; Chilli germplasm; Genetic diversity; Morphological traits

Introduction

Chilli peppers are used as vegetable and spices globally. It has been using since the beginning of civilizations. Bosland [1] reported that this ranked the chilli amongst the oldest cultivated crops of the Americas. According to Heiser [2] genus *Capsicum* is one of the first crop plants being grown in the New World with maize, beans and cucurbits. It is belonging to nightshade family *Solanaceae*, tribe Solanaceae, subtribe Capsicinae [3]. Most *Capsicum* species are 2n = 2x = 24. Some of the non-

domesticated species are 2n = 2x = 32 [4]. The names of variety depending on type and location and the common names of pepper are chilli, red pepper, green bell or only pepper [5]. It is originated from South and Central America [6].

Mexico, as the centre of domestication and genetic diversity of *Capsicum*. It is considered as the first condiment to have been utilized by human beings and there is archaeological evidence of pepper and other fossil foods from as early as 6000 years ago [7]. The pungent varieties are

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generally named chilli pepper or simply "chilli". It plays very important role in commercial sector. Specially, it is used in cosmetics, pharmaceutical industries, and for oleoresin preparation. Chilli extensively grown for making powder is also harvested green. There are many nutritional, medicinal and economic benefits of its production. It is used for salad and as condiment also. It is used in foods for pungency and red colour.

It is a small perennial shrub characterized by greenish-white corolla or white, more than one pedicel at a node having different fruit shapes and sizes [8]. Pepper grows relatively rapid with a maturity time of 3-4 months. It grown as an annual in the temperate region [9]. It grows on different types of soil but is most appropriate to welldrained loamy soils, rich in lime, with a pH of 5.5–6.8 and more water holding capacity. The optimum day temperatures for chilli pepper growth range from 20 to 30 °C [10]. It is often a cross-pollinated crop up to 63%. Pakistan is among the major chillies exporter and producer viz China, Mexico, Morocco and Turkey. In a global scenario, India is contributing (11 lac ton) 25 %. The global chilli production is approximately 7 million ton cultivated on 1.5 million hectares. Among top chilli producing and export countries, India exports 25%, China 24%, Spain 17%, Mexico 8%, Pakistan 7.2%, Morocco 7% and Turkey 5% are respectively. Badin, Mirpur khas and Umerkot, districts of Sindh represent the national clusters of red chillies production. A town of Kunri in Umerkot, contributes about 86% share in chilli production and is known as one of the leading production centers in Asia for red chillies [11]. Land of Pakistan blessed with varied types of climate and soil that grow various exogenous and indigenous medicinal plants, condiments, bearing plants and herbs. Regardless of the availability of the diverse agro-climatic conditions of the country, to produce the extensive range of plant species and as they were contributing in the national economy, by producing

significant export earnings or import exchange [12].

Phenotypic characters such as plant height, fruit shape, fruit weight and flower colour have been used to differentiate genotypes of peppers and categorize them into groups [13]. Smith and Smith [14] reported that the use of phenotypic traits in categorizing and describing of germplasm is an important step in every characterization program. Morphological and conventional markers called descriptor used for identification of variety and genetic diversity in a plant. The aim of this study was to characterize germplasm using agronomical and morphological traits. This evaluation contributes to estimate the variability of the genotypes and also to determine promising materials appropriate for hybridization programs.

Materials and methods

Material collection and site selection

This study was conducted at Bio-resources and Conservation Institute (BCI), National Agricultural Research Centre (NARC), Islamabad. The plant material comprised of about 38 genotypes of pepper including a check variety (P6). The germplasm was obtained from Bio-resources and Conservation Institute (BCI), National Agricultural Research Centre (NARC), Islamabad.

Nursery of Capsicum was raised in greenhouse at NARC. The nursery was watered daily in the morning and evening till the seed was germinated. Care was taken to avoid too much watering to prevent lodging of seedlings. Field plants were transplanted after 35 days in a field in an augmented design with a plant to plant and row to row distance 45cm and 75cm respectively. The land was prepared by ploughing two times to level the field. Cultural practices and plant protection measurements were carried out when required raising a healthy crop. After transplanting, insecticide Lambda was sprayed on the plants to guard against the attack of tobacco caterpillar. Furadon was also used to control the cut warm.

A set of parameters covering different growth stages selected from the *Capsicum* descriptors developed by Biodiversity International former [15] for recording the data on agronomic traits. Information on the phenotype given by descriptors on morphological parameters is essential in giving accurate identification of species [3]. Ten plants of each genotype were selected randomly for data recording. Fourteen morphological (qualitative) traits were studied on a visual basis as shown in (Table 1).

Data on the agronomical (quantitative) traits regarding Plant height (cm) of each pepper, plant was recorded. The 1st fruit has

begun to ripe. Mature leaf width (cm) and Leaf length (cm), leaf length was measure from base to the endpoint of the leaf whereas width was recorded at the widest point of the leaf, after maturity of the plant. Fruit width (cm) was measured at the widest point. Fruit length (cm) was recorded at the largest point. Fruit weight (g) was measured by weighing the fruits after picking. Fruit wall thickness (mm) was recorded at the second harvest at the point of maximum width. A number of locules was counted by cutting fruit at the broadest point. Fruit yield per plant (g) total fruit weight of each plant of each genotype was recorded.

 Table 1. Descriptor developed by Biodiversity International (former IPGRI, Rome) for recording the data on qualitative traits

Codes	Classes		
1, 2, 3	Annual, Biennial, Perennial		
1, 2, 3, 4	Green ,Green with purple stripes, Purple, Other		
3, 5, 7	Sparse,Intermediate, Dense		
1, 2, 3	Deltoid, Ovate, Lanceolate		
	Entire Undulate, Ciliate		
3, 5, 7	Sparse, Intermediate, Dense		
3, 5, 7	Pendant, Intermediate, Erect		
1, 2, 3, 4	White, Yellow, Green, Orange		
1, 2, 3, 4, 5, 6, 7, 8	White ,Lemon-yellow, Pale yellow, orange-yellow, Orange, Light red, Red, Dark red		
1, 2, 3, 4, 5, 6	Elongate Almost round, Triangle, Campanulate, Blocky, Other		
1, 2, 3, 4, 5, 6	Acute, Cordate, Obtuse, Lobate, Truncate		
0, 1	Absent ,Present		
1, 2, 3, 4	Straw (deep yellow) Brown, Black, Other		
1, 2, 3	Smooth, Semi wrinkled Wrinkled		
	$ \begin{array}{r} 1, 2, 3 \\ 1, 2, 3, 4 \\ 3, 5, 7 \\ 1, 2, 3 \\ 1, 2, 3 \\ 1, 2, 3 \\ 3, 5, 7 \\ 3, 5, 7 \\ 1, 2, 3, 4 \\ 1, 2, 3, 4, 5, 6 , \\ 7, 8 \\ 1, 2, 3, 4, 5, 6 \\ 1, 2, 3, 4, 5, 6 \\ 0, 1 \\ 1, 2, 3, 4 \\ \end{array} $		

Statistical analysis

Means were calculated through formula $(\sum x/n)$ for all quantitative traits. Standard error, mean, standard deviation, variance, the range was calculated through descriptive stat using Microsoft excel.

Results and discussion

Morphological characterization is a procedure, which requires a descriptive list, suggested by [16] to provide more information about gene pool preserved in gene banks and working collections.

Morphological characterization is the only way of differentiation of genotypes on the basis of physical appearance. It is also very bringing agronomically useful for important traits, especially quantitative characters for improvement of the crop [17]. However it is also vital in genotypic identification, its application is greatly affected by prevailing environmental conditions and making the results locationspecific [17, 18]. Smith and Smith [14] reported that the use of phenotypic traits in classifying and describing germplasm is rudimentary in any characterization program. All genotypes were characterized for different agro-morphological characters as shown in (Table 2). All parameters were measured in the laboratory and field at the usual harvest time.

Variability based of morphological traits Data concerning morphology about 14 qualitative traits were estimated by the following data descriptor. Qualitative characteristics were determined on visual basis. According to the data descriptor all the qualitative traits are assigned with the code, data were recorded in the form of specific codes. All 38 genotypes showed (100 %) result regarding the annual life be due might to inherent cycle. characteristics. Data regarding to the stem colour, green colour (84.21 %) was depicted in 32 genotypes. Intermediate stem pubescence was dominant in 29 genotypes with 76.31 % in frequency. Results concerning leaf shape, ovate leaf

shape (44.73 %) were recorded in 17 genotypes. Data regarding lamina margin we found 32 genotypes showed undulate lamina margin with 84.21 %. Results related leaf pubescence, as shown in (Table 2), 18 genotypes (47.36%) had sparse pubescence. Manju and Sreelathakumary [19] found sparse leaf pubescence in Capsicum chinense germplasm. Datta and Das [20] reported that maximum of the genotypes exhibited sparse leaf pubescence in *Capsicum annum*. Data regarding flower position, maximum 23 genotypes showed (63.52 %) intermediate position [21] reported that flower position in most of the genotypes, especially the wild cultivars had erect positions. Data regarding fruit colour intermediate stage, maximum at 26 genotypes depicted orange colour at intermediate stage with 68.42 % frequency. Highest 22 genotypes showed dark red colour (57.89 %) at mature stage of fruit. Elongate fruit shape was prominent in 29 genotypes with 76.39 %. Lobate fruit shape at pedicle attachment was dominant in 24 genotypes with 63.15 %. Neck at the base of fruit was present in 25 genotypes with 65.78 % frequency. Seed colour 36 genotypes (98.73 %) showed vellow colour of seed and 2 genotypes showed black seed colour might be due to immature seed extraction. Fifty percent of genotypes had smooth fruit surface, were found in 19 genotypes.

S. No.	Parameters	Frequency	% age				
1	I	Life cycle					
	Annual	38	100				
	Biennial						
	Perenial						
2	Stem color (SC)						
	1.Green	32	84.21				
	2.Green with purple stripes	2	5.2				
	3.Purple	4	10				
	4.Other	0	0				
3	Stem Pubescence (SP)						
	3.Sparse	5	13.15				
	5.Intermediate	29	76.31				
	7.Dense	4	10.52				
4	Leat	f Shape (LS)					
	1.Deltoid	16	42.10				
	2.Ovate	17	44.73				

Table 2. List of qualitative	characters recorded of	n the base of visu	al observation

-	3.Lanceolate	5	13.15
5		Margin (LM)	10.15
	1.Entire	5	13.15
	2.Undulate	32	84.21
	3.Ciliate	1	2.63
6		ibescence (PB)	15.0.4
	3.Sparse	18	47.36
	5.Intermediate	16	42.10
	7.Dense	4	10.52
7		position (FP)	
	3.Pendant	7	18.42
	5.Intermediate	23	60.52
	7.Erect	8	21.05
8		at Intermediate Stage	
	1.White	0	0
	2.Yellow	1	2.63
	3.Green	10	26.31
	4.Orange	26	68.42
	5.Purple	1	2.63
	6.Deep purple	0	0
	7.Other	0	0
9	Fruit Colou	ir at Mature Stage	
	1.White	0	0
	2.Lemon-yellow	0	0
	3.Pale orange-yellow	0	0
	4.Orange yellow	1	0
	5.Pale orange	0	0
	6.Orange	4	10.52
	7.Light red	10	26.31
	9.Dark red	22	57.89
	10 Purple	0	0
	11.Brown	0	0
	12.Black	0	0
	13.Other	0	0
10	Fruit	t Shape (FS)	
	1.Elongate	29	76.31
	2.Almost round	7	18.42
	3.Triangle	0	0
	4.Campanulate	0	0
	5.Blocky	2	5.26
11	Fruit Shape a	t Pedicel Attachment	
	1.Acute	7	18.42
	2.Cordate	2	5.2
	3.Obtuse	0	0
	4.Lobate	24	63.15
	5.Truncate	5	13.15
12	Neck at	t Base of Fruit	
	0.Absent	13	34.21
	1.Present	25	65.78
13	Seed	Colour (SC)	
	1.Straw (deep yelow)	36	94.73
	2.Brown	0	0
	3.Black	2	5.20
	4.Other	0	0
14		Surface (FC)	
		19	50
	1.51100011	17	50
	1.Smooth 2.Semiwrinkled	16	42.10

Variability based on agronomic traits

The explanation of morphological character, variation is very significant in the commencement of programs intended with of genotypes the selection having quantitative characters like yield, fruit weight, length and width of fruit. Genetic variability evaluation includes mean and range which supports in the selection of genotypes as these designate characteristic of genotypes. Mean and range for 10 quantitative traits for all set of genotypes are given in (Table 3). The magnitude of variability existing in the genotypes was measured in terms of general mean, standard deviation, range and variance. It is important character for the selection purpose. (Fig. 1) displayed variation among the genotypes regarding to fruit size shape and colour.

In present investigation, data collected regarding the plant height showed considerable differences among set of genotypes. Range of plant height varied from 40 cm to 63 cm with the mean value 52.51±2.58 as shown in (Table 3). Highest (63 cm) plant height was seen in 36645 and 36616. Lowest (40 and 41 cm) plant height was observed in 36588 and 36591 genotypes. A variety P6 showed 60 cm of plant height. Similar results were observed in investigation of [22]. The variation in growth of pepper plant is controlled by environmental factors mainly moisture, humidity, temperature, light, soil fertility and environment etc.

Leaves are the main organ for photosynthesis and play a vital role in the growth and survival of plant. Data recorded for leaf width varied from (1.10 cm to 4.50 cm) with mean value of 2.76 ± 0.15 shown in (Table 4). Maximum (4.5, 4.3, 4.3, 4.2 and 4.1cm) leaf width in overall set of genotypes was found in genotype 36636 whereas minimum (1.1 cm) was noted in 36565 genotypes. Data concerning leaf length, range of leaf length varied from (2.1 cm to 5.77 cm) with mean values 3.82±0.18. Maximum (5.7 and 5.6 cm) leaf length was seen in 36617 and 36597

genotypes whereas P6 showed (3.1 cm) of leaf length Minimum (2.1 cm) leaf length was recorded in 36651. Many previous investigations have shown that variations in leaf character are the outcome of adapting to growth habitat [23]. Our results are in accordance with [24].

Data regarding to number of locules recorded from tested cultivars range from 1.50 to 2.20 with mean value of 1.97±0.02. Maximum (2.20) number of locules was observed in 36624 followed by (2.21) was seen in 36632 genotype while minimum (1.50) was seen 36637 genotype. A variety P6 showed (2) number of locules. Lippman and Tanksley [25] reported that some varieties produced fruit with more number of locules, resulting in wider and larger fruit.

The fruit width and length are leading traits, help in differentiating the varieties as small, medium, and large size. Results obtained for fruit width range from 0.60 cm to 2.90 cm with mean value of 1.63 ± 0.11 . Maximum (2.90) fruit width was found in 36114 and 36560 genotypes. Minimum (0.60) fruit width was found in 36569. The data presented considerable variation regarding fruit length and width among the *Capsicum* genotypes as shown in (Fig. 1).

The Capsicum genotypes displayed significant variability concerning fruit length, range of fruit length varied from 2.21 cm to 13.2 cm with the mean value 7.06 ± 0.55 . Maximum (13.2 cm) fruit length was observed in genotype 36636. Minimum (2.1 cm) fruit length was observed in 36591. Enough variation was seen in length and width of fruit is due to long and thin fruits wide fruits of chillies as shown in (Fig. 1). These findings are in accordance with [26, 27] also found that pepper cultivars and varieties varied significantly in fruit length, width and weight.

Fruit wall thickness is also most influential element in yield [28]. Firm fruits are appropriate for picking, transportation, storage and marketing. It was measured in millimetre. Fruit wall thicknesss varied from (0.51 to 2.60 mm) with a mean value of 1.35±0.10. Highest (2.6mm) pericarp thickness was found in genotype 33828. Minimum (0.51) was observed in 36635 genotypes. Check variety P6 showed (1.5 mm) of fruit wall thickness.

Fruit weight is powerful characteristic for the selection process. The results of these studies demonstrated a single weight of varied among fresh fruit/plant the *Capsicum* genotypes from (0.85 g to 8.30 g)with the mean value 3.56±0.29. Maximum (8.3 g) was observed in genotype 36651, while minimum (0.85 g) fruit weight was found in genotype 36591. Wide variation related to fruit weight was recorded among due genotypes to small, medium. large/wide genotypes. Fruit weight is quantitatively hereditary trait, controlled by many genetic loci, some with a minor effect and other with large effect [29]. Fruit weight has a maximum contribution towards increase in yield/ plant.

Number of fruit also important trait, contribute to the final yield. Data regarding number of fruit varied from (37.1 to 298.0) with mean value of 148.20±9.55. Highest (298.0) number of fruit was observed in genotype 36613 while lowest (37.1) was found in genotype 36652. About (154.3)

numbers of fruit were observed in p6 variety. Our results are in accordance with [30].

Yield is a multipart trait and determined by numerous factors. The variation in quality characters and yield is determined by environmental factors and genetic of plant [31]. But yield constituents are somewhat and little affected simple by the environmental factors. Data concerning yield varied from 175.49g to 780.3 g with the mean value of 521.02±28.01. Maximum (780.3 g) yield/plant was observed in genotype 36616 while minimum (175.94 g)was seen in genotype 36652. Whereas (509.80) g yield/plant was depicted in P6. This variation was recorded for fruit length (cm), fruit weight (g), plant height (cm), and in fruit yield per plant of different Capsicum genotypes, due to their heredity characteristics and response of genotypes to the fluctuating environmental factors, fertilization, disease and pest, control management, processing and harvesting methods during growing season. Genotypes displayed great variation for all the evaluated traits, confirming the affirmation of [32].

Traits	Mean	SD	Variance	Rai	Check variety	
Traits	Mean	50	variance	Maximum	Minimum	P6
Plant height	52.51 ±2.58	8.00	64.06	63	40	60.1
Leaf width (cm)	2.76±0.15	0.93	0.86	4.50	1.10	2.83
Leaf length (cm)	3.82±0.18	1.16	1.36	5.77	2.1	3.1
Number of locules	1.97±0.02	0.15	0.02	2.20	1.50	2
Fruit width (cm)	1.63±0.11	0.69	0.48	2.90 0.60		2.6
Fruit length (cm)	7.06±0.55	3.51	12.29	13.2	2.21	9.86
Fruit wall thickness (mm)	1.35±0.10	0.63	0.40	2.60	0.51	1.5
Single Fruit weight (g)	3.56±0.29	1.67	2.81	8.30	0.85	3.38
Number of fruit/ plant	148.20±9.55	60.38	2905.29	298.0	37.1	154.3
Yield/plant (g)	521.02±28.0 1	177.14	28043.38	780.3	175.49	509.80

Table 3. Descriptive statistics for the (agronomic) quantitative traits



Figure 1. Diversity shown among pepper genotypes regarding: shape of fruit, fruit size and fruit colour at mature stage

Genoty	PH	LW	II (ana)	NOI	FW	FL	FW	FWT	SFW	NE/D	Y/P
pe ID	(cm)	(cm)	LL(cm)	NOL	(cm)	(cm)	(cm)	(mm)	(g)	NF/P	(g)
36637	43	1.8	4.6	1.5	0.98	5.1	0.98	0.67	2.59	123.1	520
36635	43	2.8	2.9	2	0.97	6.87	0.97	0.51	3.65	140.23	419.8
36620	44	1.3	5	2	1.7	4.7	1.7	1.5	4.6	191.34	566.75
36588	40	2.8	4.4	2	1.3	3.6	1.3	0.66	1.45	100.23	398.7
36622	49.23	4.3	2.5	2	1.1	4.9	1.1	1	1.84	87.4	286.5
36624	60.23	1.2	2.7	2.2	1.65	8.87	1.65	1.3	3.85	156.6	699.5
36636	62	4.5	4.6	2	1.97	13.2	1.97	2.3	7.45	200.45	777.1
36592	60.13	2.9	3.2	2	1.72	9.21	1.72	0.93	3.69	165.44	645.2
36597	60.45	3.3	5.6	1.7	1.35	5.8	1.35	1.2	3.4	164.8	579.43
36591	41	1.9	2.3	2	2.48	2.1	2.48	1.9	0.85	174	250.3
36632	46	1.5	2.2	2.2	0.8	5.6	0.8	0.9	3.7	219.8	660.34
36617	61	4.2	5.6	2	0.7	2.8	0.7	1.1	2.6	218	561.922
36623	45	1.4	2.8	2	1.5	4.5	1.5	0.77	1.56	194.3	475.8
36616	63	3.9	5	2	1.7	3.1	1.7	1.1	2.4	298	780.3
36628	57	4.3	5.3	1.7	1.2	5.8	1.2	1.2	2.8	278.9	715.47
36612	59	2.5	2.1	2	1.5	6.3	1.5	1.6	3.18	99.3	423.9
36114	60	2.2	5.4	2	2.9	7.43	2.9	2.5	4	142.5	567.34
36649	47	3.7	4.2	1.8	1	6.7	1	1	3.87	145	230
30881	45.12	3.1	4.2	2	0.7	3.7	0.7	0.66	1.5	206.6	360.55
36570	61	2.1	3.3	2	1	7.2	1	1	4	107.4	629.23
36569	46	2.7	5.1	2	0.6	3.8	0.6	0.7	1.4	149.2	420.71
36627	47	2.1	3.1	2	2.5	12.0	2.5	2.4	7.2	79.82	509.8
30879	58	2.4	5.1	2	1.4	5.4	1.4	0.98	2.33	128.9	500.12
36565	59.23	1.1	2.3	2.1	2.82	8.57	2.82	1.3	3.55	147.67	635.4
36560	60	2.8	3.1	1.9	2.9	9.24	2.9	0.9	3.65	154	698.2
33828	55	4.1	5.3	2	1.8	11.8	1.8	2.6	6.5	148	667.8
36563	61	3	3.2	2	1.7	7.3	1.7	2.2	3.5	152.3	572.594
36644	58	3.5	4.2	2	1.4	8.1	1.4	1.4	4.56	123.4	612.4

Table 4. Means values of agronomic (quantitative) parameters

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36645	63	2.2	4.1	1.9	1.3	5.3	1.3	1.6	2.67	164.4	625.43
36658	57	3.7	2.4	2	1.3	6.9	1.3	0.67	2.87	156.7	531.43
36630	47	1.9	4.3	2	1.7	4.5	1.7	0.8	2.09	100.34	194.3
30877	49	3	2.9	2	2.7	11.2	2.7	2.3	5.7	97	703.4
36651	42	3	2.1	2	2.8	10.9	2.8	1.9	8.30	98	713.5
36652	42	3.5	5.2	2	1.7	8.54	1.7	1.5	4.19	37.1	175.94
36558	46	2.5	4.6	2	1.6	5.6	1.6	1.93	3.98	49.5	194.57
36564	48	3.2	2.5	2	1.23	7.6	1.23	1.98	3.27	137	476.44
36656	49	2	4.9	2	1.6	7.6	1.6	0.69	3.1	140.2	498.7
P6	60.1	2.8	3.1	2	2.6	9.86	2.6	1.5	3.38	154.3	509.80

Plant height: PH, Leaf width, LW leaf length: LL, Number of Locules: NOL, Fruit width: FW, Fruit wall thickness: FWT, Single fruit weight: SFW, Number of fruit per plant: NF/P, Yield per plant: Y/P

Conclusion

Chilli is an essential cash crop, cultivated for spices and food all over the world. It is also used in medicines with lots of benefits to human health. Estimating unique genotypes in this study it was possible to genetic variation find immense at phenotypic level in Capsicum genotypes in climatic condition of Pakistan. We have selected 4 genotypes 36616, 36613, 36636 and 36560 on the basis of best performing morphological characters among whole set of genotypes characters These genotypes are potential source of genes for breeding programs for development of cultivar for food consumption.

Authors' contributions

Conceived and designed the experiments: N Khan & MJ Ahmed, Performed the experiments: N Khan, Analyzed the data: N Khan, Contributed materials/ analysis/ tools: T Shehzad, M Ahmed, S Bashir & A Hamid, Wrote the paper: N Khan.

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