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ASSESSMENT OF GENETIC DIVERGENCE AND ITS UTILIZATION IN HYBRID DEVELOPMENT IN CULTIVATED ONION (*ALLIUM CEPA* L.)

R. Ahmad[1*], M. K. N. Shah[1], Mahmood-ul-Hassan[1], D. Ibrar[1], R. A. Javaid[1] and N. Khan[2]

[1]Department of Plant Breeding and Genetics, PMAS-Arid Agriculture University, Rawalpindi, Pakistan
[2]Magnus Kahl Seeds (MKS) Pty Ltd. 6A Dairy Drive, Coburg North, Victoria 3058, Australia
[*]Corresponding Author's Email: rafiqahmad.uaar@gmail.com

ABSTRACT

Exploration of genetic variation may help to select diverse parents for open pollinated variety and hybrid development program in onion (*Allium cepa* L.). Thirty-five onion genotypes were analyzed by two-way cluster analysis and principal component analysis (PCA) on the basis of phenotypic traits. These genotypes were grouped in five different clusters. PCA provided information for variation in genotypes on the basis of studied traits. Ten genotypes were selected from five diverse clusters and hybridized in a line × tester mating fashion. Twenty-four F_1 combinations were developed and raised along-with parents for assessment of hybrid vigor, commercial heterosis and genetic correlation. Data was analyzed for morphological and biochemical traits. Maximum commercial heterosis (heterosis computed over commercial standard/check variety) for bulb yield per plot was observed in combination MKS-SGB × MKS-GBP-01, MKS-57404 × Super Sarhad and MKS-SGB × Mustang (55.77%, 36.70% and 32.90%), respectively. Maximum better parent heterosis for bulb yield per plot was in combinations MKS-SGB × MKS-GBP-01 (31.01%), followed by MKS-TEG × Mustang, MKS-57404 × Super Sarhad and MKS-SGB × Mustang (13.76%, 12.49% and 11.79%), respectively. Genetic correlation coefficient showed that bulb yield per plot showed highly significant and positive association with single bulb weight, bulb diameter, significant positive association with leaf density (number of leaves per plant), leaf width (diameter) and significant but negative association with total soluble solids. Yield per plot showed a negative association with days to maturity. Present study revealed that cluster analysis and PCA can play an important role in effective parent selection without compromising genetic diversity for development of potential hybrids for commercial exploitation.

Key words: *Allium cepa*, Hybrid development, Commercial heterosis, Cluster analysis, Principal component analysis, Correlation.

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INTRODUCTION

Onion (*Allium cepa* L.) belongs to the largest genus of cultivated monocot (*Allium*), which is comprised of about 750 species (Stearn, 1992). Onion is one of the most important vegetable crops ranked second in production after tomato throughout the world (Pathak, 2000; Mallor *et al*., 2011). Onion is being consumed in variety of ways throughout the world because of its nutritional and medicinal value (Dhanya *et al*., 2014). Onion has a considerable contribution in export and shares major part by vegetables export in economy of the country (Karar *et al*., 2013). Pakistan ranks 3rd in area under cultivation of the crop after India and China, while at 5th in production and at 90th in yield per acre (FAOSTAT, 2018).

Genetic variability for different economic and consumers preference traits (bulb yield per plot, bulb shape, bulb colour and bulb hearting) is available in germplasm of bulb onion, but progress in crop improvement is quite slow because of genome complexity (McCallum, 2007; Varshney *et al*., 2012). Consumers and growers preferences play an important role in selection of genotypes by breeders for hybrid and open pollinated (OP) variety development. Therefore, the genotypic selection by breeders can vary according to the preference of growers and consumers (Mallor *et al*., 2011). Genetic variability always plays a decisive role for making an improvement in cross and self pollinated crop species (Griffing and Lindsstromm, 1954). Studies to explore genetic variability among existing germplasm and selection of diverse and suitable parents become very important for any successful plant breeding program.

Cluster Analysis is a useful tool for grouping of genotypes in clusters on the basis of weighted means and provides a way for breeders in selection of parents (Dangi *et al*., 2018). Principal component analysis (PCA) is one of the important methods from multivariate analysis by which breeder can identify key traits responsible for variation among genotypes (Kovacic, 1994).

Contribution of improved open pollinated varieties (OPVs) and hybrids almost doubled the production potential of cultivated onion in last five decades (Brewster, 2008). The increase or decrease in the vigor of F_1 hybrids and proceeding generations with respect to their parents is chiefly associated with heterotic effect (Abdullah *et al*., 2002). Considerable heterosis was estimated for bulb weight, variation for bulb colour in different hybrid combinations (Jones and Davis, 1944). The F_1 hybrid showing high heterotic effect may yield a desirable OPV in proceeding generations as compared to the F_1 showing low heterotic effect (Sharif *et al*., 2001).

The reason of low yield of the country may be attributed largely to lack of improved varieties and hybrids under cultivation. In Pakistan decades old OPVs are being cultivated whereas little OPV and no heterosis breeding program is in place. Further, mass selection is the predominant variety improvement method in onion (González, *et al*., 2012) which leads to little improvement in productivity coupled with narrowing the genetic base. Not much emphasis was given on selection of better parents for OPV development and heterosis breeding except some characterization work (Mushtaq *et al*., 2013). Present study was aimed to assess the genetic variability of available germplasm, selection of suitable parents and development of hybrids. It may provide a basic platform for the improvement of onion yield of the country.

MATERIALS AND METHODS

Plant Material: Thirty-five onion genotypes collected by Magnus Kahl seeds (MKS) including some local genotypes were utilized in this study (listed in Table S1). These genotypes were evaluated in field area of department of plant breeding and genetics, PMAS Arid Agriculture University, Rawalpindi in 2015-2016. Nursery was grown in month of October and transplanted in end of December with three replications by keeping all standard field experimental protocol and agronomic practices required at different stages of experiment. Data were collected for the traits like germination percentage, leaf length, leaf width, leaf density (Number of leaves per plant), neck diameter, bulb yield (kg / plot (m^2), bulb colour, bulb shape and bulb hearting.

On the basis of PCA (Hotelling, 1933) and Cluster analysis (Zubin, 1938) (Heat map cluster was constructed), selection of suitable parents was carried out for hybrid development.

Hybrid Development: Parents selected (Table 4) were crossed in a line × tester mating fashion producing twenty-four F_1 hybrid combinations during 2016-17. For female parents five plants were planted in one row keeping P-P distance of 45 cm and adjacent row of male parent with three plants by keeping inter row distance of two feet in a cage to maintain isolation. Seeds harvested from each combination were sown in next season (2017-18) for heterotic response analysis as described by Sharma and Singh (1978). The following traits were studied for evaluation of F_1 combinations. Experiment was carried out by using twenty four F_1 combinations, ten parents and one check (Phulkara) variety in three replications following randomized completed block design (RCBD). Plot area for each genotype was $1m^2$ with plant to plant distance of 10 cm and row to row distance of 25 cm.

Agronomic traits: Five plants from each replication were randomly selected to collect data for each parameter at its proper stage. Germination percentage was studied by germinating seeds. Hundred seeds were sown in a plastic pot for each genotype separately with three repetitions. Leaf length was measured with a meter rod and leaf diameter (width) with vernier caliper at around 50% bulb formation. Neck diameter (mm) was measured when 50% plant attain maturity with the help of Vernier calipers (Mitutoyo Corporation, Japan; model # CD-8″). Days to maturity was calculated by counting days from sowing to 50% neck fall. Single bulb weight was calculated by randomly selected bulbs and averages were taken in grams. Bulb diameter (mm) was measured with vernier caliper. Dry matter parentage was computed by taking fresh bulb weight and dry weight (after drying the chopped onion at 60° C for 48 hours or until further no change in dry weight). Dry matter percentage was calculated by using following formula;

$$\text{Dry matter percentage} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

Qualitative traits: Bulb colour and shape were scored by using *Allium* plant descriptor by International Plant Genetic Resources Instit., (IPGRI), Rome (Italy) published in 2001. Bulb hearting (centers) was also counted according to this descriptor by cutting the bulb in cross sections and counting its hearts or centers.

Biochemical traits: Biochemical traits *viz.,* total soluble sugars (TSS) and Pyruvic acid concentrations were measured. TSS were measured by hand held Refractometer (model # LH-T80). A drop of onion juice extracted by common blender was dropped on a prism of Refractometer and results were expressed in Brix°. Pyruvic acid, considered as the chief determinant in onion pungency, was estimated by the classical method proposed by Schwimmer and Weston (1961) further repeated by Yoo *et al.,* 1995.

Statistical Analysis: Data collected was subjected to Analysis of variance, Cluster analysis, PCA, heterosis, heterobeltiosis, commercial (useful) heterosis and genotypic correlation estimate analysis. ANOVA of RCBD design was performed as mentioned in the sub-section “Agronomic traits” of Materials and Methods. Level of significance was calculated by checking the F_{cal} value. All the statistical data analysis was performed using software R-Studio version 3.5.1.

RESULTS

Morphological Variability: The germplasm showed considerable variability for the traits studied in this experiment (Table 1). All the traits showed significant results at 1% level of significance except bulb yield per plot that showed significance at 5% level of significance. The results showed that germination percentage ranged from 98.3% to 25.8%. Maximum germination percentage (98.33% and 96.67%) was recorded in MKS-SGB and MKS-57165, respectively. Leaf length varied from 69.3 cm to 29.5 cm. Maximum leaf length was calculated as 69.3 cm in MKS-012. Leaf density ranged from 13.4 leaves per plant to 5.8 leaves per plant. Maximum leaf density (number of leaves per plants) was observed as 13.4 leaves per plant in genotype Nasarpuri and minimum leaf density was 5.8 leaves per plant in genotype MKS-57165. Leaf width or diameter ranged from 15.3 mm to 8.9 mm which was maximum in Mustang (15.3 mm) and minimum in genotype MKS-RI-001 (8.9 mm). Neck diameter varied between 14.1 mm to 8.7 mm. Maximum neck diameter was observed in genotype MKS-SGB (14.14 mm) and minimum neck diameter was observed in MKS-RI-001 (8.71 mm). Bulb yield per plot ranged from 3.8 kg per plot (m^2) to 2.0 kg per plot (Table S2). Maximum bulb yield per plot was 3.80 kg per plot in MKS-57404 while minimum weight was 2.03 kg per plot in genotype MKS-503.

Table 1. Genotypic mean square for different morphological traits, grand mean, range and coefficient of variation (CV %) among different onion genotypes.

Traits	MSg	Grand Mean	Range	CV%
Germination %age	1082.18**	67.3	98.3 - 25.8	13.79
Leaf length (cm)	246.49**	47.1	69.3 - 29.5	7.82
Leaf density (number of leaves per plant)	10.04**	8.49	13.4 - 5.8	8.04
Leaf Width (mm)	11.01**	11.4	15.3 - 8.9	12.08
Neck diameter (mm)	6.18**	11.2	14.1 - 8.7	8.69
Bulb yield (kg/plot)	0.31*	3.0	3.8 - 2.0	4.13

MSg = Genotypic mean squares, ** = Highly significant at 1 %, * = Significant at 5 %
CV% = Coefficient of variation

Cluster Analysis: Thirty-five genotypes were analyzed for hierarchical cluster analysis (HCA). The dendrogram generated by UPGMA cluster analysis for phenotypic traits separated thirty five genotypes into five major clusters; these are I, II, III, IV and V clusters (Table 2). Cluster heat map analysis (Fig. 1) was also carried out for these genotypes. Heat map presented here provides the two-way classification between genotypes and traits and provided an opportunity to visualize and observe the expression of each trait. The colour scheme in this figure showed the magnitude of trait relatedness to genotypes that ranged from dark red (maximum association of traits) to dark blue (minimum contribution of traits). Classification of the traits in such way allows an effective overview and enables to select the genotypes for breeding program according to required traits from different groups. Considerable diversity was observed for the traits studied in the germplasm. Maximum of thirteen genotypes were found in cluster III, cluster V consisted of nine genotypes, cluster I consisted of five genotypes and cluster II and IV consisted of four genotypes each.

Principal Component Analysis (PCA): The PCA exhibited the importance of major contributor towards total variation explained by each axis of differentiation (Sharma, 1998). The PCA identified four PCs with eigen value > 1, which explained about 78.78% of the total genetic variation in studied germplasm (Table 3). The first PC explained 33% of the total variability (Fig. 2). From the present results, first principal component showed high and positive component values for leaf density (number of leaves per plant), neck diameter and bulb yield per plot. Negative values against component 1 were exhibited for germination percentage and bulb colour. Genotypes with maximum germination percentage, more bulb hearting and dark bulb colour contributed towards second principal component. The traits showing high positive to negative values revealed more genetic variability in germplasm. Hanci and Gokce (2015) explained that PCA simplifies complex data from number of variables that are correlated to smaller variables known as principal components (PCs). First component always contributed maximum variability whereas second component with lower variability. Genotypes Desi Red, MKS-501, MKS-108 and MKS-012 showed deviation from all other genotypes and formed their own group (Fig. 2).

Parent Selection for F_1 Combination Development: After screening of germplasm through different biometrical approaches and multivariate analysis, ten genotypes with best performance for bulb yield and quality attributes (Table 4) were selected. Maximum varieties (5) were selected from cluster I which comprised mainly of high yielding varieties.

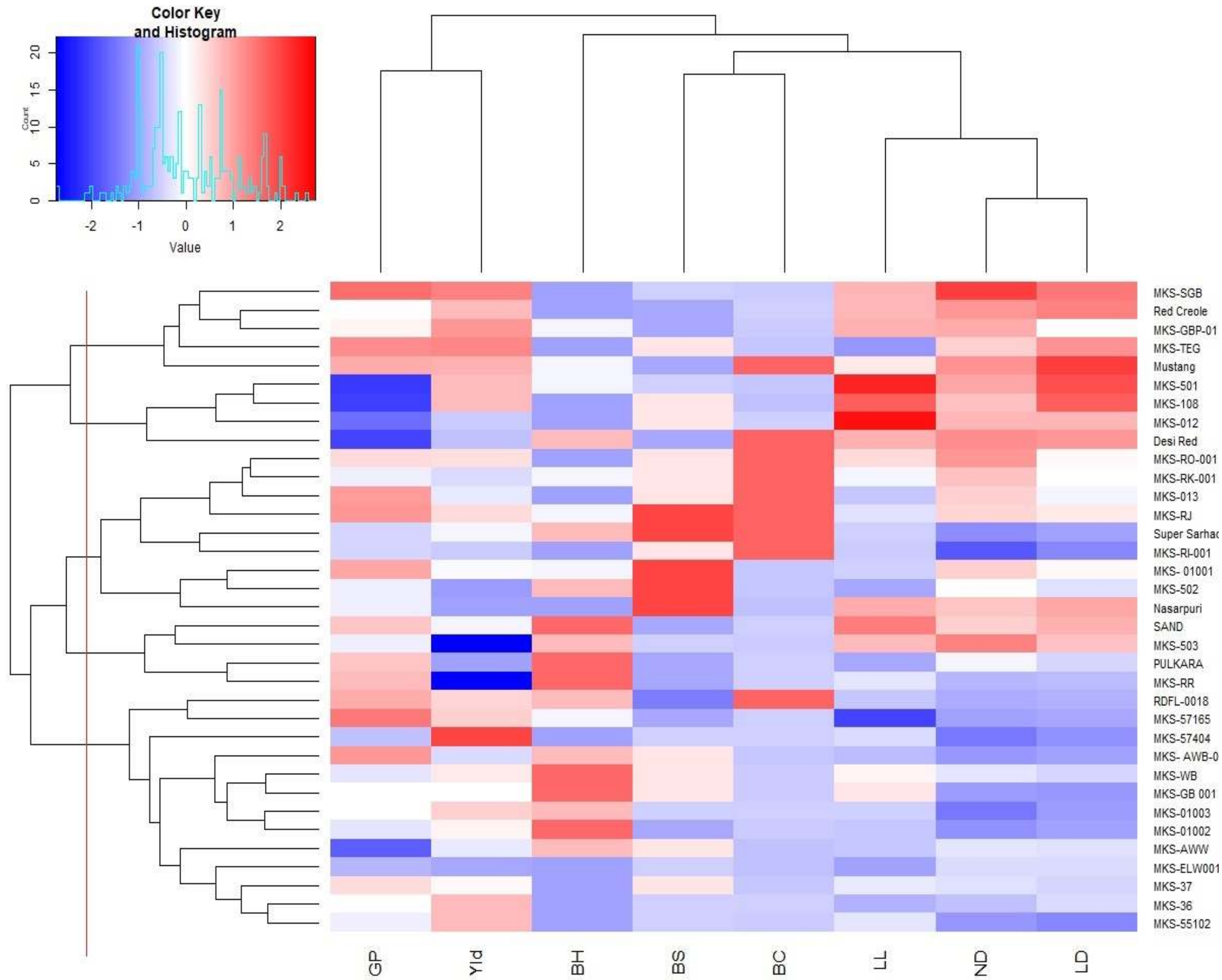

Fig. 1. Cluster heat map generated by UPGMA for the diversity of thirty five onion genotypes and some morphological and bulb traits with corresponding expression of genotype against each trait. LD; leaf density, ND; neck diameter, LL; leaf length, BC; bulb colour, BS; bulb shape, BH; bulb hearting, Yld; bulb yield (kg / plot), GP; germination percentage

Table 2. Distribution of thirty-five onion genotypes in five different clusters.

Sr. No.	Clusters	No. of Genotypes	Name of genotypes
1	I	5	Mustang, Red Creole, MKS-GBP-01, MKS-SGB, MKS-TEG
2	II	4	Desi Red, MKS-501, MKS-108, MKS-012
3	III	13	MKS-57165, MKS- AWB-002, MKS-57404, MKS-55102, MKS-GB001, MKS-RF-001, MKS-01002, MKS-01003, MKS-AWW, MKS-ELW001, MKS-36, MKS-37, MKS-WB
4	IV	4	Phulkara, SAND, MKS-RR, MKS-503
5	V	9	MKS-RK001, MKS-RO-001, Super Sarhad, MKS-RI-001, MKS-01001, MKS-RJ, Nasarpuri, MKS-013, MKS-502

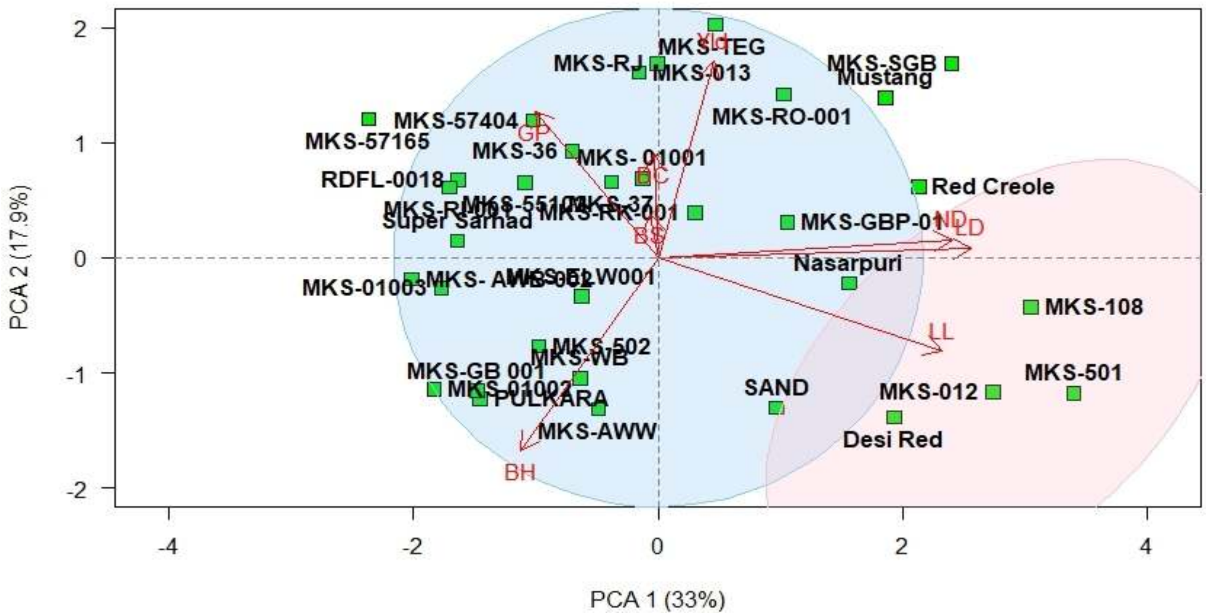

Fig. 2. Principal Component Analysis for some morpoloical and Bulb traits in onion genotypes. LL; leaf length, LD; leaf density, ND; neck diameter, BC; bulb colour, BS; bulb shape, BH; bulb hearting, Yld; bulb yield (kg / plot), GP; germination percentage.

Table 3. Eigen values, variance percentage and cumulative percentage of variance generated by PCA phenological traits in onion genotypes.

PC	Eigen value	% Variance	Cumulative Percentage
1	2.6428	33.04	33.035
2	1.4298	17.87	50.91
3	1.1905	14.88	65.79
4	1.0392	12.99	78.78
5	0.8568	10.71	89.49
6	0.4984	6.23	95.72
7	0.2460	3.08	98.79
8	0.0965	1.21	100

Table 4. List of selected genotypes with salient features and cluster number used in L x T mating design for F_1 hybrid development in onion during 2016-17.

Genotype Code	Genotype Name	General Characters Used in Selection of Parents	Cluster Number
Lines (Female Parent)			
V-1	MKS-57165	Bulb shape (Flat globe), bulb colour (Pink)	III
V-8	MKS-57404	High yield (3.8 kg / plot), bulb shape (Rhomboid)	III
V-16	MKS-SGB	High yield (3.55 kg / plot), bulb shape (Rhomboid)	I
V-17	MKS-TEG	Good yield (3.55 kg / plot), bulb shape (Globe)	I
Testers (Male Parent)			
V-2	Mustang	Bulb weight (155 g), good yield (3.55 kg / plot)	I
V-6	Super Sarhad	Bulb colour (Red), bulb shape (Globe top shaped)	V
V-11	Red Creole	Bulb shape (Flat Globe), bulb colour (Red)	I
V-14	SAND	Bulb colour (Light red)	IV
V-15	MKS-GBP-01	Good yield (3.55 kg / plot), bulb shape (Flat Globe)	I
V-27	MKS-108	Bulb colour (White), bulb shape (Globe)	II

Heterosis (mid parent), commercial parent (useful heterosis) and heterobeltiosis (better parent heterosis) for morphological, biochemical and bulb attributes: The results for heterosis over mid parent and better parents for twenty four F_1 hybrids are presented in Table 5a and 5b. Heterosis over commercial parent (useful heterosis) was calculated by using Phulkara as a commercial check. The range varied for different traits from negative to positive heterosis over mid and better parent. Commercial heterosis for bulb yield per plot ranged from maximum (55.77%) in MKS-SGB × MKS-GBP-01 to minimum (-39.41%) in MKS-57165× Red Creole. Heterosis over mid parent for bulb yield per plot ranged from -33.6% (MKS-SGB × MKS-GBP-01) to -43.79% (MKS-57165 × Red Creole). Maximum heterosis over mid parent bulb yield per plot was observed in combinations MKS-SGB × MKS-GBP-01 (33.6%), MKS-57404 × Super Sarhad (21.2%) and MKS-SGB × Mustang (17.76%). Heterosis over better parent (heterobeltiosis) for bulb yield per plot ranged from 31.01% (MKS-SGB × MKS-GBP-01) to -46.22% (MKS-57165× Red Creole. Maximum heterobeltiosis was observed for F_1 combination MKS-SGB × MKS-GBP-01 (31.01%), MKS-TEG × Mustang (13.76%) and MKS-57404× Super Sarhad (12.49%) (Table 5a).

Great variation for mid parent, better parent heterosis (heterobeltiosis) and commercial heterosis was observed for single bulb weight. Maximum commercial heterosis for single bulb weight was observed in combination MKS-SGB × MKS-GBP-01 (73.86%) followed by MKS-57404 × Super Sarhad (45.08%) and MKS-SGB × Mustang (36.89%) as indicated in Table 5a.

Range of commercial heterosis for bulb diameter varied from 38.03% to -22.8% in combination MKS-SGB × Mustang and MKS-57165 × Mustang respectively. Maximum commercial heterosis for bulb diameter was calculated in combinations MKS-SGB × Mustang (38.03%) followed by MKS-SGB × MKS-GBP-01 (29.02%) and MKS-SGB × SAND (28.28%). Maximum heterosis over mid parent was observed in MKS-SGB × Mustang (30.21%), MKS-SGB × MKS-GBP-01 (15.21%) and MKS-TEG × Mustang (14.17%).

Commercial heterosis for leaf length ranged from 24.39% for combination MKS-57165 × Super Sarhad to -22.95% for combination MKS-TEG x Super Sarhad**.** Heterosis over mid parent for leaf density (number of leaves per plant) varied from -30% in hybrid combination MKS-TEG x SAND to 12.38% and10% in MKS-57404 x MKS-GBP-01. While, heterosis over better parent (heterobeltiosis) for leaf density (number of leaves per plant) ranged from -30% in combination MKS-TEG x SAND to 10.0% in combination MKS-57404 x MKS-GBP-01 (Table 5b).

Mid parent heterosis for leaf diameter (leaf width) ranged between -24.90% for hybrid combination MKS-TEG × Super Sarhad to 37.84% in combination MKS-SGB × MKS-GBP-01. While, heterobeltiosis for leaf diameter ranges from -31.20% in combination MKS-TEG × SAND to 33.98% in combination MKS-SGB × MKS-GBP-01. Heterosis over mid parent for neck diameter ranged between -49.13 (MKS-TEG × Super Sarhad) to 49.56 (MKS-TEG × Super Sarhad). Mid parent heterosis for dry matter percentage ranged from -62.88% for combination MKS-SGB × MKS-GBP-01 to 108.87% in combination MKS-57404× MKS-108. While heterosis over better parent (heterobeltiosis) ranged from -65.29% MKS-SGB × MKS-GBP-01 to 81.07% in combination MKS-57404× MKS-108 as indicated in Table 5b**.**

Range for mid parent heterosis for total soluble solids varied from -23.32% (MKS-57165× Super Sarhad**)** to 38.95% (MKS-57404 × MKS-GBP-01). Heterosis over better parent (heterobeltiosis) for total soluble solids varied from -28.15% in MKS-57165× Super Sarhad to 25.71% in hybrid combination MKS-57404 × MKS-GBP-01. Mid parent heterosis for pyruvic acid percentage varied from -25.84% (MKS-SGB × MKS-GBP-01) to 147.12% (MKS-SGB × Mustang). Heterosis for days to maturity ranged from -10.19% for hybrid combination MKS-57165 × MKS-108 to 13.92% for combination MKS-TEG × Super Sarhad. Better parent heterosis for days to maturity ranged from -15.83% for hybrid combination MKS-57165 × MKS-108 to 13.13% for combination MKS-TEG × Super Sarhad (Table 5b)**.**

Genetic Correlation Analysis for Yield and Contributing Traits: Results for Genetic Correlation were computed for twenty-four F_1 hybrids and ten parental genotypes that are presented in Table S3. Correlation coefficient (r) is a measurement of association between different qualitative and quantitative traits. In case of onion it shows the association of morphological characters with bulb characters. Trait associations are presented in Fig. 3. In this figure size of block shows magnitude of trait association and colour of box shows range from unit positive (dark blue) to unit negative (dark red). Bulb yield per plot showed highly significant and positive association with single bulb weight (0.98^{**}), bulb diameter (0.87^{**}), leaf length (0.62^{**}) and leaf density (0.37^{*}). Single bulb weight showed significant association with bulb diameter (0.87^{**}) and leaf length (0.63^{**}). Leaf density showed highly positive association with leaf length (0.72^{**}), bulb diameter (0.44^{**}). Leaf width showed significant positive correlation with dry matter percentage (0.40^{*}).

Table 5a. Mid parent (MPH), commercial (CH) and better parent (BPH) heterosis for leaf length, bulb diameter, single bulb weight and bulb yield per plot of twenty four F_1 hybrid combinations.

Combination	Leaf Length			Bulb Diameter			Single Bulb Weight			Bulb yield per Plot		
	MPH	BPH	CH	MPH	BPH	CH	MPH	BPH	CH	MPH	BPH	CH
MKS-SGB × Super Sarhad	-23.99**	-28.03**	-0.68^{ns}	-4.46^{ns}	-4.78^{ns}	8.39*	3.58^{ns}	0.95^{ns}	17.50*	-0.72^{ns}	-6.96^{ns}	10.71^{ns}
MKS-SGB × MKS-GBP-01	-6.15*	-13.93**	18.71**	15.21**	13.34**	29.02**	56.98**	49.38**	73.86**	33.60**	31.01**	55.77**
MKS-SGB × SAND	-4.77^{ns}	-15.20**	17.03**	11.45**	10.20**	28.28**	17.31**	8.14^{ns}	25.87**	10.29*	1.45^{ns}	20.70**
MKS-SGB × MKS-108	-13.66**	-23.64**	5.39^{ns}	-6.13*	-6.39^{ns}	7.11*	-2.88^{ns}	-3.60^{ns}	12.21*	-9.80*	-11.11*	5.72^{ns}
MKS-SGB × Red Creole	-12.31**	-22.77**	6.51^{ns}	-14.00**	-19.55**	-8.45*	-20.10**	-22.90**	-10.25*	-27.05**	-28.99**	-15.44*
MKS-SGB × Mustang	-10.37**	-15.32**	16.83**	30.21**	21.31**	38.03**	19.05**	17.61**	36.89**	17.76**	11.79**	32.90**
MKS-57404× Super Sarhad	-5.53^{ns}	-10.48**	10.43*	3.20^{ns}	-1.16^{ns}	22.06**	22.58**	14.95**	45.08**	21.2**	12.49**	36.70**
MKS-57404× MKS-GBP-01	3.78^{ns}	1.66^{ns}	17.05**	1.23^{ns}	-4.25^{ns}	18.23**	10.45*	1.22^{ns}	27.75**	-0.10^{ns}	-3.03^{ns}	17.76**
MKS-57404× SAND	-15.98**	-16.98**	-8.40*	2.42^{ns}	-0.51^{ns}	22.81**	12.79*	0.27^{ns}	26.55**	7.79^{ns}	-1.80^{ns}	19.24**
MKS-57404× MKS-108	0.37^{ns}	-1.59^{ns}	8.69*	-24.88**	-27.63**	-10.61*	-24.80**	-28.20**	-9.40^{ns}	-27.93**	-29.71**	-14.63*
MKS-57404× Red Creole	-11.96**	-14.09**	-1.94^{ns}	3.65^{ns}	-6.58*	4.02^{ns}	-9.99*	-16.40**	0.51^{ns}	1.72^{ns}	-1.99^{ns}	9.10^{ns}
MKS-57404× Mustang	-29.73**	-33.27**	-18.03**	0.73^{ns}	-9.56**	11.70**	-19.30**	-23.30**	-3.25^{ns}	-20.18**	-24.98**	-8.84^{ns}
MKS-57165× Super Sarhad	12.60**	0.84^{ns}	24.39**	-6.63*	-9.62**	9.21*	8.25^{ns}	6.49^{ns}	17.67*	7.22^{ns}	6.63^{ns}	10.87*
MKS-57165× MKS-GBP-01	-22.13**	-28.07**	-17.27*	-17.24**	-20.9**	-4.46^{ns}	-17.56**	-18.30**	-12.60*	-24.13**	-27.94**	-17.65*
MKS-57165× SAND	3.73^{ns}	-1.18^{ns}	6.50^{ns}	-10.85**	-12.47**	5.75^{ns}	2.50^{ns}	-1.68^{ns}	5.12^{ns}	-2.27^{ns}	-3.69^{ns}	-0.95^{ns}
MKS-57165× MKS-108	-1.21^{ns}	-5.19^{ns}	0.54^{ns}	0.80^{ns}	-1.86^{ns}	18.54**	9.29^{ns}	5.58^{ns}	21.09**	7.47^{ns}	1.59^{ns}	17.43*
MKS-57165× Red Creole	-5.51^{ns}	-8.88*	-4.30^{ns}	-23.22**	-30.11**	-15.60**	-40.19**	-40.50**	-35.70**	-43.79**	-46.22**	-39.41**
MKS-57165× Mustang	-19.04**	-27.36**	-10.79*	-29.47**	-36.06**	-22.80**	-35.79**	-37.70**	-29.21**	-36.33**	-37.53**	-33.30**
MKS-TEG × Super Sarhad	-38.98**	-40.32**	-22.95**	-25.52**	-25.66**	-15.60**	-36.08**	-38.90**	-25.88**	-33.66**	-34.48**	-30.16**
MKS-TEG × MKS-GBP-01	-20.85**	-25.11**	-3.47^{ns}	-22.02**	-23.18**	-12.80**	-16.62**	-22.2**	-5.55^{ns}	-19.50**	-22.21**	-11.01^{ns}
MKS-TEG × SAND	-26.89**	-32.90**	-13.47*	-21.83**	-22.81**	-10.20**	-22.55**	-29.9**	-14.95*	-22.31**	-24.78**	-19.86**
MKS-TEG × MKS-108	-13.88**	-21.52**	1.29^{ns}	-13.46**	-13.82**	-1.39^{ns}	-5.90^{ns}	-8.51^{ns}	11.10^{ns}	-5.85^{ns}	-9.45*	4.68^{ns}
MKS-TEG × Red Creole	-8.59**	-17.07**	6.93^{ns}	-2.59^{ns}	-8.76**	3.52^{ns}	-7.29^{ns}	-12.38*	6.40^{ns}	-8.6-*	-11.02*	0.25^{ns}
MKS-TEG × Mustang	-1.84^{ns}	-4.21^{ns}	23.56**	14.17**	8.37*	22.99**	6.18^{ns}	2.74^{ns}	29.03**	13.89**	13.76**	21.57**

Table 5b. Mid parent and better parent heterosis for leaf density, leaf diameter (width), neck diameter, dry matter, total soluble solids, pyruvic acid percentage and days to maturity of twenty four F_1 hybrid combinations.

Combination	Leaf Density (number of leaves per plant)		Leaf Diameter (width)		Neck Diameter		Dry Matter Percentage		Total Soluble Solids		Pyruvic Acid Percentage		Days to Maturity	
	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH
MKS-SGB × Super Sarhad	-15.64**	-20.00**	6.81^{ns}	-2.18^{ns}	2.59 ^{ns}	-23.46**	-6.70^{ns}	-30.59**	-7.32**	-19.49**	101.27**	80.22**	11.93**	9.09**
MKS-SGB × MKS-GBP-01	8.36*	2.76^{ns}	37.84**	33.98**	24.34 **	7.72^{ns}	-62.88**	-65.29**	3.12^{ns}	-5.71*	-25.84**	-40.30**	4.80**	4.51*
MKS-SGB × SAND	-20.59**	-26.21**	24.05**	14.49*	49.56 **	23.2**	-12.48*	-36.09**	0.00^{ns}	-13.45**	86.23**	72.82**	7.42**	4.83**
MKS-SGB × MKS-108	-18.46**	-26.90**	7.78^{ns}	0.39^{ns}	39.60 **	35.67**	-19.98**	-34.24**	0.49^{ns}	-12.71**	6.25^{ns}	4.70^{ns}	7.25**	6.20**
MKS-SGB × Red Creole	5.66^{ns}	-3.45^{ns}	-11.37^{ns}	-21.83**	34.23 **	9.79*	15.96**	-13.47**	19.17**	8.49**	108.72**	98.67**	13.77**	12.36**
MKS-SGB × Mustang	3.20^{ns}	0.00^{ns}	13.60*	0.43^{ns}	7.46 ^{ns}	-18.5**	23.07**	-12.10**	-1.45^{ns}	-15.00**	147.12**	130.95**	-3.93*	-7.04**
MKS-57404× Super Sarhad	-5.38^{ns}	-5.38^{ns}	29.74**	29.56**	-1.45 ^{ns}	-15.28**	-45.78**	-57.80**	-13.3**	-25.42**	83.06**	73.66**	0.65^{ns}	-1.78^{ns}
MKS-57404× MKS-GBP-01	12.38**	10.00*	5.70^{ns}	-0.48^{ns}	29.48 **	25.20**	72.49**	71.82**	38.95**	25.71**	9.32*	1.65^{ns}	8.85**	3.31*
MKS-57404× SAND	-24.62**	-24.62**	3.99^{ns}	-6.86^{ns}	-7.95 ^{ns}	-10.40*	-33.65**	-49.41**	6.86**	-8.40**	2.91^{ns}	-5.89^{ns}	-7.83**	-10.18**
MKS-57404× MKS-108	3.67^{ns}	-2.31^{ns}	36.44**	27.28**	45.19 **	25.22**	108.87**	81.07**	-3.45^{ns}	-16.95**	-6.38^{ns}	-18.98**	-0.40^{ns}	-6.11**
MKS-57404× Red Creole	0.80^{ns}	-3.08^{ns}	-8.54^{ns}	-9.55^{ns}	-16.95 **	-19.88**	99.44**	55.71**	13.09**	1.89^{ns}	29.13**	5.97^{ns}	-0.92^{ns}	-4.58**
MKS-57404× Mustang	-6.77^{ns}	-8.82^{ns}	-7.36^{ns}	-8.63^{ns}	5.95 ^{ns}	-7^{ns}	-12.36*	-34.77**	-4.39^{ns}	-18.33**	24.61**	0.74^{ns}	8.97**	0.51^{ns}
MKS-57165× Super Sarhad	5.43^{ns}	0.77^{ns}	16.17**	9.11^{ns}	6.00 ^{ns}	-11.19**	-32.58**	-32.71**	-23.32**	-28.15**	-16.68**	-19.28**	-2.33^{ns}	-5.28**
MKS-57165× MKS-GBP-01	-7.82^{ns}	-10.04*	12.03^{ns}	6.06^{ns}	4.00 ^{ns}	3.70^{ns}	62.81**	27.22**	9.17**	-2.96^{ns}	2.55^{ns}	-11.64*	9.45**	3.27*
MKS-57165× SAND	-4.23^{ns}	-8.46^{ns}	-0.87^{ns}	-5.91^{ns}	31.10**	23.83**	9.93*	6.64^{ns}	0.79^{ns}	-5.19*	22.53**	21.63**	-1.95^{ns}	-5.03**
MKS-57165× MKS-108	-10.92*	-12.24*	3.96^{ns}	-0.65^{ns}	-1.59 ^{ns}	-12.81*	23.23**	8.51^{ns}	-11.46**	-17.04**	1.14^{ns}	-5.49^{ns}	-10.19**	-15.83**
MKS-57165× Red Creole	-11.95**	-12.5*	-2.38^{ns}	-11.80^{ns}	-9.73 *	-15.47**	-29.94**	-30.13**	2.07^{ns}	-8.89**	-4.94^{ns}	-16.24*	1.84^{ns}	-2.51^{ns}
MKS-57165× Mustang	-19.06**	-24.26**	-5.29^{ns}	-14.23*	-15.42 **	-27.7 **	-36.46**	-40.44**	-12.16**	-17.04**	36.38**	18.18**	5.48**	-3.27*
MKS-TEG × Super Sarhad	-26.15**	-26.15**	-24.90**	-28.90**	-49.13 **	-51.62**	-5.69^{ns}	-7.10^{ns}	3.57^{ns}	-1.69^{ns}	-20.36**	-31.22**	-0.55^{ns}	-2.67^{ns}
MKS-TEG × MKS-GBP-01	10.02*	7.69^{ns}	-8.39^{ns}	-14.02*	20.86 **	5.45^{ns}	33.78**	3.27^{ns}	6.16*	5.66*	-17.62**	-19.99**	13.92**	13.13**
MKS-TEG × SAND	-30.00**	-30**	-22.9**	-31.20**	-28.64 **	-34.25**	-60.46**	-61.00**	-12.89**	-17.65**	78.15**	48.92**	2.33^{ns}	0.27^{ns}
MKS-TEG × MKS-108	-3.67^{ns}	-9.23^{ns}	18.10**	9.82^{ns}	8.53 ^{ns}	-14.35**	72.54**	49.72**	0.89^{ns}	-4.24^{ns}	-15.90**	-33.05**	11.05**	9.50**
MKS-TEG × Red Creole	-10.40*	-13.85**	-17.50**	-18.70**	-18.74 **	-24.49**	-45.15**	-46.21**	-5.66*	-5.66*	27.02**	-3.48^{ns}	6.93**	6.04**
MKS-TEG × Mustang	-4.51^{ns}	-6.62^{ns}	-22.70**	-24.00**	-24.85 **	-26.84 **	-49.69**	-52.07**	-5.31*	-10.83**	31.36**	-1.49^{ns}	2.32^{ns}	-1.40^{ns}

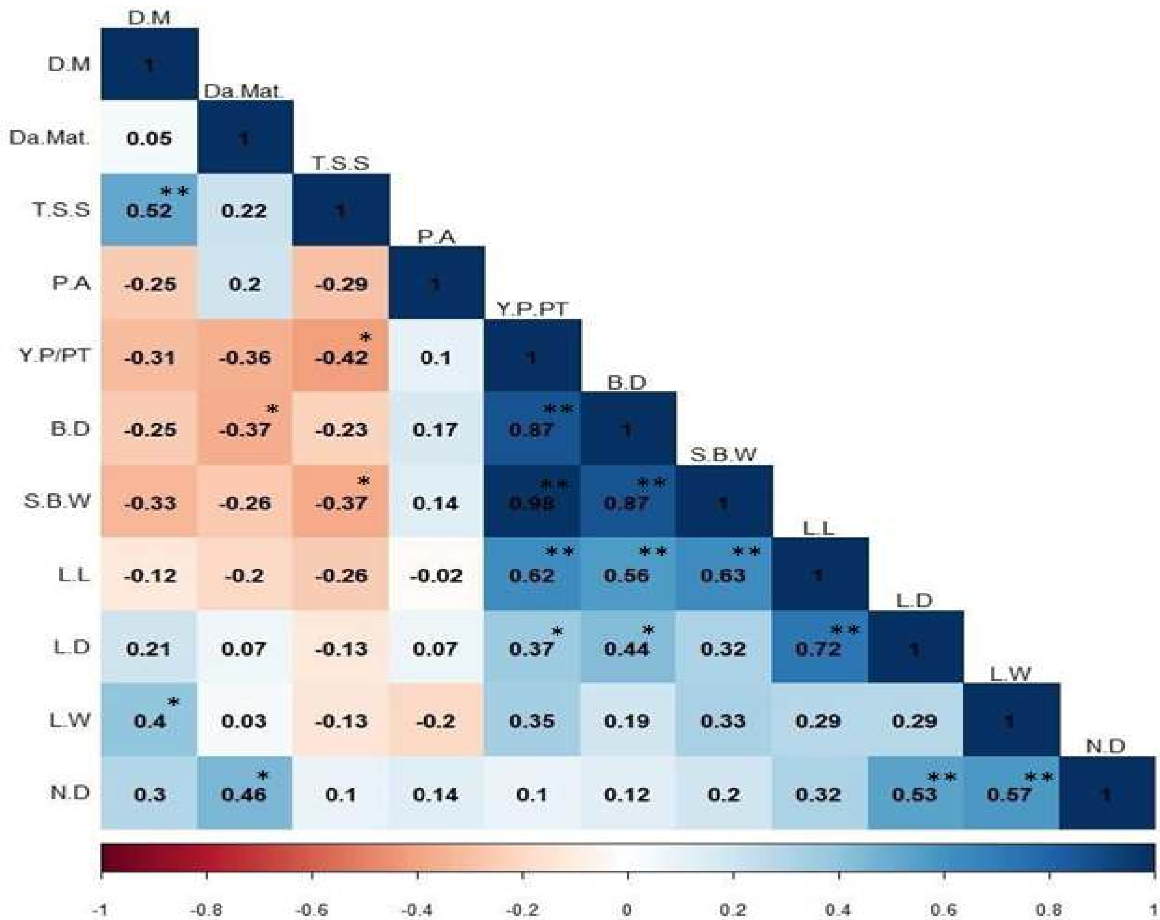

Fig. 3. Genetic Correlation analysis for morphological, Bulb and biochemical traits in onion. ND; neck diameter (mm), LL; leaf length (cm), LW; leaf width (mm), LD; leaf density (number of leaves per plant) SBW; single bulb weight (g), BD; bulb diameter (mm), TSS; Total Soluble Solids (brix°), Y.P/PT; Bulb yield per plot (kg), DM; dry matter %, PA; pyruvic acid%, Da.Mat.; days to maturity

DISCUSSION

Onion breeding in Pakistan is a neglected avenue. Farmers are forced to rely on decade old OPVs (developed through mass selection) or imported hybrid seed that has lesser adaptability over the environments. Contemporary onion growing countries have long ago shifted to hybrid onion, thus, getting quite high yields.

Enriching the gene pool with diverse genotypes is the foremost and first important step towards efficient onion breeding. Identification of suitable diverse parents is the other requirement for OPV and hybrid onion development program with broad genetic base. In current study ten genotypes have been identified with high yield potential as well as desired consumer and grower's preferences with broad genetic base. Genotypes were selected from diverse clusters (groups) on the basis of bulb yield per plot (MKS-57404 produced 3.80 kg per plot, MKS-SGB produced 3.55 kg per plot and MKS-TEG produced 3.53 kg per plot) bulb shape (MKS-57165 (flat globe), Red Creole (flat globe) and Super Sarhad (globe top shaped) and colour (Super Sarhad (red), MKS-57165 (pink)). Cluster analysis is one of the important approaches of multivariate analysis for study diversity and grouping of genotypes into clusters on the basis of similarities/differences among them. It has also been regarded as an efficient tool of varietal classification (Tsukazaki *et al.,* 2010). Arya *et al.* (2017) studied diverse onion genotypes for morphological and bulb traits and found great diversity. They indicated that genotypes, from different clusters which are important for bulb and its yield contributing traits, can be used for future breeding program. Jankulovska *et al.* (2014) also emphasized that extant of variation in breeding material can be seen clearly. They also mentioned that this technique might be useful for selecting genotypes at early breeding stage. Lee *et al.* (2015) while studying organic and conventional onion observed that first three PCs contributed 77.41% to 84.71% of the total variation. The results for principal component analysis are supported by the studies of Hanci and Gokce (2015). Similar studies were conducted by Singh *et al.* (2013) for different morphological, biochemical and bulb traits in onion who found similar results. The results of presented studies are also in accordance with the studies of Sudha *et al.* (2019). Arya *et al.* (2017) also studied different morphological and bulb traits for diversity analysis through principal component analysis and found that maximum traits were highly positive contributor towards total variation studied in onion. Present results for genetic correlation of single bulb weight or bulb yield per plant to bulb diameter and bulb yield per plot are supported by the results of Raghuwanshi *et al.* (2016); Saini and Maurya (2014) and Awale *et al.* (2011). Results for leaf density or number of leaves per plant to neck diameter and number of leaves per plant to leaf length were supported by the findings of Raghuwanshi *et al.* (2016) and Dhall and Brar (2013).

These selected genotypes MKS-SGB, MKS-57165, MKS-TEG, MKS-57404 and Super Sarhad) that produced good hybrid combinations can easily provide a basis for onion improvement and hybrid development program. Considerable variation was found for heterosis (mid and better parent) from low to high for different traits studied from morphological to biochemical traits. Some combination showed highest values for single bulb weight, bulb diameter and bulb yield per plot. From present results some combinations showed negative heterosis for days to maturity which is an indicator of early maturity, thus revealing that crosses with negative heterosis for days to maturity will take less time to achieve physiological maturity than their parents. Our results are supported by the findings of Gulumbe *et al.* (2018). Results for bulb yield were supported by the studies of Pavlović *et al.*(2015). From the present results of heterosis and heterobeltiosis, four combinations were observed with maximum values for bulb yield and its contributing traits. Similar results were obtained by Farid *et al.* (2012) who observed that these kinds of combinations with most of the desirable traits can be used in future hybrid development program. Kant *et al.* (2011) studied heterosis and heterobeltiosis in cross combinations of winter and spring wheat. They indicated that this kind of study can create an ample scope for exploitation of heterosis for yield and its contributing traits.

Combinations MKS-SGB × MKS-GBP-01, MKS-57404× Super Sarhad and MKS-SGB × Mustang can be used for commercial hybrid production and OPV development because traits of local market requirements are available. Efficient hybrid breeding program may require identification of Cytoplasmic male sterile lines and maintainer lines for commercial hybrid seed production. Advances in molecular techniques may help to identify such lines. Combinations developed in present study revealed significant potential for utilization in onion improvement program.

Conclusions: Present investigations provided the considerable information for genotypic selection, their performance in F_1 and selection of best possible combinations. It is further suggested that parents (MKS-SGB, MKS-57165, MKS-TEG, MKS-57404 and Super Sarhad) of such combinations may be utilized for haunting CMS and maintainer lines using conventional and molecular approaches.

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Supplementary Material

Table S1. Onion germplasm used for screening of morphological and bulb traits.

Sr. No.	Code	Genotype Name	Source	Sr. No.	Code	Genotype	Source
1	V-1	MKS-57165	MKS	19	V-19	MKS-01002	MKS
2	V-2	Mustang	MKS	20	V-20	MKS-01003	MKS
3	V-3	PULKARA	MKS	21	V-21	MKS-AWW	MKS
4	V-4	MKS-RK-001	MKS	22	V-22	MKS-ELW001	MKS
5	V-5	MKS-RO-001	MKS	23	V-23	MKS-RR	MKS
6	V-6	Super Sarhad	MKS	24	V-24	MKS-RJ	MKS
7	V-7	MKS- AWB-002	MKS	25	V-25	Desi Red	MKS
8	V-8	MKS-57404	MKS	26	V-26	MKS-501	MKS
9	V-9	MKS-55102	MKS	27	V-27	MKS-108	MKS
10	V-10	MKS-GB 001	MKS	28	V-28	MKS-012	MKS
11	V-11	Red Creole	MKS	29	V-29	MKS-503	MKS
12	V-12	RDFL-0018	MKS	30	V-30	Nasarpuri	MKS
13	V-13	MKS-RI-001	MKS	31	V-31	MKS-013	MKS
14	V-14	SAND	MKS	32	V-32	MKS-36	MKS
15	V-15	MKS-GBP-01	MKS	33	V-33	MKS-37	MKS
16	V-16	MKS-SGB	MKS	34	V-34	MKS-WB	MKS
17	V-17	MKS-TEG	MKS	35	V-35	MKS-502	MKS
18	V-18	MKS- 01001	MKS				

Tables S2. Mean values of genotypes for germination percentage, leaf length, leaf width, neck diameter, leaf density and yield (Kg/plot).

Genotypes	Germination (%)	Leaf Length (cm)	Leaf Width (mm)	Neck Diameter (mm)	Leaf density (Number)	Bulb Yield (kg/plot)
MKS-57165	96.67^{ab}	29.5^{m}	9.6^{klmn}	9.75^{fghij}	5.8^{o}	3.25^{def}
Mustang	85.00^{abcde}	49.3^{efgh}	15.3^{a}	12.92^{ab}	9.1^{efg}	3.38^{bcd}
Pulkara	80.00^{cdef}	38.8^{kl}	10.5^{ijklmn}	11.04^{cdefg}	7.1^{jklmn}	2.68^{n}
MKS-RK-001	63.33^{ghij}	45.9^{ghij}	$11.3^{fghijklm}$	12.22^{bcde}	9.0^{efg}	2.90^{jklm}
MKS-RO-001	75.00^{defg}	50.7^{efg}	$11.5^{efghijkl}$	12.81^{ab}	9.7^{de}	3.18^{defgh}
Super Sarhad	58.33^{hij}	42.4^{ijkl}	9.6^{klmn}	9.45^{ghij}	7.0^{klmn}	3.00^{hijkl}
MKS- AWB-002	90.00^{abcd}	40.8^{jkl}	9.5^{klmn}	9.64^{fghij}	7.1^{jklmn}	2.90^{jklm}
MKS-57404	53.3^{ij}	43.7^{hijk}	9.2^{mn}	9.14^{hij}	7.4^{ijklmn}	3.80^{a}
MKS-55102	63.33^{ghij}	44.5^{hijk}	9.0^{n}	9.65^{fghij}	7.5^{ijkl}	3.33^{cde}
MKS-GB 001	68.33^{fghi}	49.5^{efgh}	9.3^{lmn}	9.69^{fghij}	7.7^{hijkl}	3.05^{fghij}
Red Creole	68.33^{fghi}	54.1^{def}	14.0^{abcd}	12.84^{ab}	9.7^{de}	3.33^{cde}
RDFL-0018	85.00^{abcde}	41.5^{jkl}	9.8^{jklmn}	9.91^{fghij}	10.7^{cd}	3.23^{defg}
MKS-RI-001	58.33^{hij}	42.1^{jkl}	9.0^{n}	8.71^{j}	7.5^{ijklm}	2.83^{klmn}
SAND	80.00^{cdef}	59.4^{cd}	$13.1^{abcdefg}$	11.96^{bcde}	9.4^{ef}	3.00^{hijkl}
MKS-GBP-01	70.00^{efgh}	54.6^{de}	$11.4^{efghijklm}$	12.49^{bc}	9.1^{efg}	3.48^{bc}
MKS-SGB	98.33^{a}	54.2^{def}	14.2^{abc}	14.14^{a}	8.2^{ghij}	3.55^{b}
MKS-TEG	91.67^{abc}	37.3^{l}	13.6^{abcde}	11.97^{bcde}	8.4^{fghi}	3.53^{bc}
MKS- 01001	86.67^{abcd}	42.8^{ijkl}	$11.6^{efghijk}$	11.97^{bcde}	8.7^{efgh}	3.03^{ghijk}
MKS-01002	61.67^{ghij}	41.5^{jkl}	9.5^{klmn}	9.52^{ghij}	6.6^{lmno}	3.10^{fghij}

MKS-01003	68.33^{fghi}	42.7^{ijkl}	9.4^{klmn}	9.12^{ij}	6.3^{no}	3.25^{def}
MKS-AWW	32.50^{l}	41.5^{jkl}	$10.8^{ghijklmn}$	10.82^{defg}	6.9^{klmn}	2.95^{ijkl}
MKS-ELW001	51.67^{jk}	38.5^{kl}	10.6^{ijklmn}	10.64^{efghi}	7.1^{klmn}	2.70^{mn}
MKS-RR	81.67^{bcdef}	44.3^{hijk}	10.1^{jklmn}	10.08^{fghij}	7.7^{hijkl}	2.03^{o}
MKS-RJ	90.00^{abcd}	43.9^{hijk}	$11.9^{defghij}$	11.89^{bcde}	7.6^{hijkl}	3.20^{defgh}
Desi Red	27.50^{l}	54.5^{de}	13.5^{abcdef}	12.96^{ab}	11.1^{c}	2.80^{lmn}
MKS-501	25.83^{l}	67.5^{ab}	15.0^{ab}	12.56^{abc}	12.2^{b}	3.33^{cde}
MKS-108	26.67^{l}	62.1^{bc}	14.7^{abc}	12.22^{bcde}	11.1^{bc}	3.33^{cde}
MKS-012	36.67^{kl}	69.3^{a}	$12.9^{bcdefgh}$	12.36^{bcd}	11.3^{bc}	2.83^{klmn}
MKS-503	63.33^{ghij}	53.6^{def}	$12.7^{cdefghi}$	13.11^{ab}	10.7^{cd}	2.03^{o}
Nasarpuri	63.33^{ghij}	55.1^{de}	$13.3^{abcdefg}$	12.15^{bcde}	13.4^{a}	2.68^{n}
MKS-013	88.33^{abcd}	41.6^{jkl}	$11.2^{ghijklmn}$	11.92^{bcde}	7.3^{ijklmn}	2.95^{ijkl}
MKS-36	66.67^{fghij}	39.9^{jkl}	10.6^{ijlmn}	10.22^{fghij}	7.5^{ijkl}	3.33^{cde}
MKS-37	75.00^{defg}	45.2^{ghij}	10.6^{ijklmn}	10.72^{efgh}	7.3^{jklmn}	3.08^{fghij}
MKS-WB	61.67^{ghij}	48.3^{fghi}	10.6^{ijklmn}	10.82^{defg}	7.8^{hijk}	3.15^{efghi}
MKS-502	63.33^{ghij}	38.6^{kl}	$10.8^{hijklmn}$	11.17^{cdef}	6.4^{mno}	2.65^{n}
LSD	15.12	6.00	2.25	1.58	1.11	0.20

Table S3. Genetic correlation coefficient for different phenological traits for F_1 combinations in cultivated onion.

Traits	LD	ND	LL	LW	SBW	BD	TSS	YPPT	DM	PA
ND	0.54^{**}									
LL	0.72^{**}	0.32								
LW	0.29	0.57^{**}	0.29							
SBW	0.32	0.2	0.63^{**}	0.33						
BD	0.44^{*}	0.14	0.56^{**}	0.19	0.87^{**}					
TSS	-0.13	0.08	-0.26	-0.14	-0.37^{*}	-0.22				
YPPT	0.37^{*}	0.1	0.62^{**}	0.35	0.98^{**}	0.87^{**}	-0.42^{*}			
DM	0.21	0.29	-0.12	0.40^{*}	-0.33	-0.25	0.52^{**}	-0.31		
PA	0.07	0.14	-0.02	-0.19	0.14	0.17	-0.29	0.11	-0.25	
Da.Mat	0.21	0.46^{*}	-0.12	0.39^{*}	-0.33	-0.25	0.52^{**}	-0.31	0.99^{**}	-0.25

Genetic Correlation analysis for morphological, Bulb and biochemical traits in onion. **ND;** neck diameter (mm), **LL;** leaf length (cm), **LW;** leaf width (mm), **SBW;** single bulb weight (g), **BD;** bulb diameter (mm), **TSS;** total soluble solids (brix˚), **YPP;** yield per plot (kg), **DM;** dry matter %, **PA;** pyruvic acid%, **Da.Mat;** days to maturity