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Research Article

Post-Harvest Storage Quality of Onion F1 Hybrids Produced Using Cytoplasmic-Genic Male-Sterility

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Abstract

Cytoplasmic-genic male (c-gms) sterility is one of the most genetically viable mechanisms of earliness, uniform bulb yield and quality in onion. Our present study was conducted in replicated and randomised block design for a period of three years to reveal the potential of three c-gms lines: Pusa Red msms, 75 msms and (102-1 x 106) msms used in producing superior onion F_1 hybrids when compared to better parent (BP), mid parent (MP), standard check (SC) and top parent (TP). Out of 60 hybrids studied, 54 hybrids were identified with heterotic bulb yield, bulb size as well as with better storage quality during five months of storage. Out of 36 superior F_1 hybrids over BP, the F_1 hybrid "75 msms x New collection from Daultabad-1 ⊗ P_2" gave maximum heterosis (85.80%) for bulb yield over BP (36.82%) and SC (113.73%). This F_1 hybrid also recorded maximum heterosis over BP (81.23%), standard check (63.58%) and top parents (34.04%). The dominant gene from female parents lead the positive directional heterosis for yield when crossed even with heterozygous testers with recessive gene at that specific location for this trait in onion genome. This was explained by tester "Arka Kalyan⊗P_1" due to observed good combining ability with three cgms lines to produce heterotic F_1 hybrids towards bulb yield irrespective of the gi and sij effects observed. The superior F_1 hybrid from cgms line "102-1x106" with tester "Red Creole –1 ⊗ P_2" was identified with early maturity of 113 days after transplanting onion seedlings. The hybrid combinations viz., "Pusa Red msms x 45-6-2-1 ⊗ P_1", "(102-1x106)msms x 103 - 3 # ⊗ P_1", "75msms x13- 50 Non-bolters from October planting # -2 #" and "(102-1x106)msms x 14 - 4 ⊗ P_4" are observed with good negative sca effects for neck diameter. The Pusa Red msms (cgms line 1), 75 msms (cgms line 2) and cgms line 3 "(102-1x106)" contributed greater percentage of negative heterosis for both neck diameter and storage loss in bulb weight. The hybrids of Pusa Red msms with tester "Agrifound Dark Red –1 –2 ⊗ P_2" followed by tester "113-1-1 ⊗ P_3" were the best combinations for total soluble solids and dry matter, with high sca effects and due to high gca effects of atleast one parent, showing their superiority over SC, BP, TP, and MP. The storage quality is mainly dependant on the genotype-environment interaction, the physiological age of the bulbs after harvest, the post harvest storage and packing quality. Identification of molecular and biochemical markers for physiological maturity, bulb scale color and related biochemicals, bulb scale thickness, thin neck and base structure may help to identify the onion germplasm with good storage quality traits.

Keywords: Cytoplasmic-genic male sterility, bulb quality, inheritance, additive or non additve gene action

Abbreviations

ANOVA: Analysis of Variance; ANCOVA: Analysis of Covariance; BP: Better Parent; CD: Critical Difference; ms: Male Sterility; cms or c-gms: Cytoplasmic-Genic Male Sterility; cm: Centimeter; cv.: Cultivar; df: Degrees of Freedom; *et al.* : co-workers; msl: Mean Sea Level; F_1: First Filial Generation; gca: General Combining Ability; g: Gram; kg: Kilogram; MP: Mid Parent; ms: Male Sterile; MSS: Mean Sum of Squares; OPV: Open Pollinated Variety; OP: Open Pollinated; RH: Relative Humidity; SC: Standard Check; sca: Specific Combining Ability; SSC: Soluble Solid Content; TP: Top Parent; TSS: Total Soluble Solids; Vs: Versus; *Viz.*: Namely; %: Percent; #: Number; x: Mating Cross; ⊗ : Selfing; °C: Degrees Celsius; °F: Degrees Fahrenheit.

Introduction

The onion (*Allium cepa* L., shallot, top set onion, multiplier onion), is the most common vegetable of the genus: *Allium*, subfamily: *Allioideae*, and the family: *Amaryllidaceae* (*Alliaceae*). This family includes other vegetables like garlic (*A. sativum*), great head leek or elephant garlic (*A. ampeloprasum*), chives (*A. schoenoprasum*), welsh onion (*A. fistulosum*), chinese chive (*A. tubersoum, A. chinense*) and beltsville bunching onion (*A. cepa* x *A. fistulosum*). Onion is an outcrossing diploid (2n = 2x = 16) that was observed with severe inbreeding depression. It is mostly cultivated as an annual to produce edible bulbs (seed to bulb) in tropics, as a biennial or a perennial by means of its bulbs or bulbils (bulb to seed or seed to seed) in regions of mild to over winter climate for seed production. Flowering (bolting) occurs after vernalization, a period of exposure to 5 - 10°C for 4 to 6 weeks. Optimum temperatures for growth and development of onion are 13 to 25°C [1-3].

Maharashtra is the biggest producer of onions in India and Lasalgaon – Nashik District is the largest hub of onions in Asia which contributes 80 per cent of the total onion export of the country. Total area under onion cultivation reduced from 10.87 to 9.58 lakh hectares in 2019. Onions are the second most valuable produce of the world where India stands second in production after China. The demand for high quality onions is always increasing due to global culinary requirements and economic significance in the world trade. The quality of onions is highly influenced by its genotype. Identification of, potential genotypes with, cytoplasmic male sterility from open pollinated varieties of onion has laid the path for production of F_1 hybrid seed in onion (Table 1 and Table 2). The male sterile line 13-53 was first identified from the populations of onion cv. Italian Red grown in California in 1927 which lead to first F_1 hybrid in onion in 1936 by Jones and Emsweller. As early as in 1943, cytoplasmic-geneic male sterility (S msms) was identified in onion for the first time by Jones and Clarke. From introduction of USSR, Turkey, India, Japan, Syria, Korea, France, Holland, New Zealand, UK and South Africa [4] as well as from Japan, France, UK, Poland, Czechoslovakia, Bulgaria and Egypt ms lines were isolated [5] during the period of 1960 to 1970.

The male sterile and maintainer lines were identified in a short day onion cultivar, Pusa Red in the Division of Vegetable Crops at Indian Agricultural Research Institute (IARI) in New Delhi [6,7]. A male sterile AC-26 line was identified from introductions of India, Bangladesh, Brazil and Philippines by Warid and Loaiza in 1996 [8]. The polygeneic inheritance due to three recessive genes: an independent gene "a" and two complementary genes, "b and c" (m_1m_1 and m_2 m_2) in conjunction with sterile cytoplasm "S" or "T" were identified in onion. The fertility restoring lines with "msms" in the classical "S" cytoplasm were maintainers of "T" cytoplasm and many European cultivars have high frequencies of these sterility alleles and low frequencies of "T" cytoplasm allowing such lines to be converted to male sterile equivalents [9-11].

Table 1: Frequency of occurance of male-sterile plants in onion. **Source:* Main source for the references in the third column is [8], though some are listed in the literature cited.

Onion Cultivars	Percent male sterile plants	Source
Scott Country globe	1	Peterson and Foskett (1953), USA.
Red Wethersfield	1	Banga and Petiet (1958), Canada
Primeur and Wijbi	1	Banga and Petiet (1958), Holland
Zittanel Gelbe	1 to 2.9	Kobabe (1958)
Pukekohe, Long, Keeper	-	Yen (1959), Newzeland
Arzamas, Russian cultivars	33.33	Markov (1960); Kazakova and Gikalo (1967)
Doratov di Parma	<0.1	Pienaar (1958)
	5 to 25	Berninger (1976)
	Frequency of "ms" gene (%)	
Commercial cultivars	86	Little., *et al*. (1994), USA
Rijnsburger and North Holland Straw Yellow	96	Van der Meer and Van Bennekom (1971), USA
Behairy	73	El-Shafie and El-Kafory (1977), Egypt
Zittauer, Vsetatska, Gros Plat d'Itale and Makowski	Moderately high	Van der Meer (1977), Egypt
Wolska, Australian Brown, Pukekohe Long Keeper and Giba 6	Moderately low	Van der Meer (1977), Egypt
Strigunovskii and Terekhovskii Mestnyi	100	Devvatov (1977), USSR

Table 2: Hybrids produced in onion globally as of to date.

*Source: Main source for the references in the third column is [8], though some are listed in the literature cited.

Note: BP: Better Parent; MS or M or ms: male-sterile; N:male-fertile; SI:Self-incompatible.

Parents of F_1 or Cross	Unique Feature of F_1 hybrid with its name if available	Source or Reference
Valencia x Ebenzera	Early Maturity	Kazakova (1963)
Oriental x Odonoletnij Sibirkij	20 to 35% increase in bulb yield	Kazakova (1963)
Spasskii x Vishenskii	Out-yielded its parent Spasskii by 85 %	Evtikhevich (1969)
Kaba 58 x Ispanskii 313	Heterotic F_1s	Popov (1975)
Kaba 1/105 x Magnifico; Johnson $4S_4$ x Abundance; Johnson $4S_4$ x Gibrid 15 $_{4-2}$	Heterotic F_1s	Kazakova., *et al*. 1978
M3 x Juene hatif extra	F_1 with 27 % heterotic increase in bulb yield	Buchavarov, 1976
M20 x Samovosdska Kaba	F_1 with 41.8% heterotic increase in bulb yield	Buchavarov, 1976
No information on parents	F_1 hybrid "Rannii" with maturity in 110 days; F_1 hybrid "Record-2" with maturity in 120 days;	Buchavarov., *et al*. 1978
ms1 x S of Mstera	No influence of pollinator in heterotic potential of 164 to 173 % in F_1 hybrids produced from this cross	Ershov and Vorob'eva, 1979
ms lines as female parents	"Prospero" an F1 with high marketable yield	Dowker., *et al*. 1984
SA 1865 (s line of Strigunoskii) x Jasenichizuti	38.8 % heterosis over BP with average bulb weight of 79 g	Panajotovic., *et al*.,1992
ms line "4A" x Zhou 2-2; ms line "4A" x Long 6-1	25% and 84% increases respectively in their bulb yields	Zhang., *et al*. 1995
Yellow x Purple	Intermediate dry matter content in F_1 hybrids	Poljanskij, 1963
No information on parents	High yielding F_1 hybrids "Warsa" and "181". F_1 hybrid "Dona" – Long periods of storage life as well a growing season required. F_1 hybrids "169 and 173" - tight skinned bulbs with good keeping quality	Scweiguth (1974)
CS 856 x CO-1; CS 665-51 x CO-1	Aggregatum onion (*Allium cepa* L var aggregatum Don.) F_1 hybrids	Pandian and Muth-ukrishnan, 1979
AC-863 x 874; AC-863 x 873	F_1 hybrids of aggregatum onion up to 30 % heterosis over BP	Vadivel., *et al*. 1981 a,b
N2-4-1 x Bellary Red; Junagadh Red x N 53; and Bellary Red x N 53	Bulb yields with 98, 96 and 81 % heterosis respectively.	Madalgiri and Bojappa, 1987
Selection 102-1 x Selection126; [(Selection 126 x Punjab Selection) x (Selection 102-1 x Selection 134)]; and Selection 96 x Punjab Selection	25.18 to 28.8 % heterosis over BP	Netra Pal, 1988
MS 65 x SI 12-1-1-1 (Hybrid1) MS 48 x SI 14-1-1-1 (Hybrid 2)	Hybrid 1 and Hybrid 5 with 40 to 50 t/ha bulb yield	Pathak and Gowda, 1994

The exotic male sterile lines were found unsuitable in short photoperiodic environments, where indigenous sources were identified in Nasik White Globe at Indian Institute of Horticulture Research (IIHR) during heterosis breeding program of onion. The male sterile plants had anthers with a translucent, green appearance in contrast to normal, dark green anthers. They indicated the possibility of strong cytoplasmic factor(s) controlling the male sterility. They isolated both male sterile and maintainer lines and suggested their use in hybrid onion production [12]. This male sterility was transferred to six genotypes initially and made 75 crosses out of which two hybrids namely "Hybrid-1" (MS 65 x SI 13-1-1-1) and "Hybrid-5" (MS 48 x SI 14-1-1-1) were released for

commercial use with high bulb yield (45-50 t/ha) and with good quality bulbs [12,13]. These F_1 hybrids were highly advantageous over open pollinated varieties (OPV) in producing onions with high quality, uniform maturity, earliness and at less cost [14-16].

The F_1 hybrid evaluation is essential to understand the combining ability of parents for unique traits and inheritance of the traits possessing high diversity among the parents for useful agronomic, morphological and biochemical traits. The analysis of the onion hybrids and vast majority of OPVs was done every year at various research locations in India and world wide [17] (Table 2). The assessment of genetic diversity is important pre-requisite for selection of potential parental combinations with high yield and quality as well as to reduce the cost of hybrid seed production because it eliminates the undesirable crosses during hybridization program [16].

Onion is highly cross pollinated crop where hybrid vigour is possible through breeding by emasculation and hand pollination until the identification of male sterile female lines. Use of cms lines improved the seed set and seed quality that directly control the bulb quality. The F_1 hybrids possessing superior traits with improved production are readily accepted by onion growers. In India, short day onions are under cultivation till today as F_1 produced until 2000s were from short day onion cultivars though, there is vast collection of germplasm from world wide to explore and exploit heterosis to benefit different diurnal temperature requirements of India.

Several scientists in onion heterosis breeding identified cultivars with greater storage life of 5- 8 months after harvest [18-23]. Post-harvest storage studies on F_1 onions was a major contribution of work done at IARI, New Delhi since 1900's. Superiority of F_1 hybrids for processing and storage qualities was observed besides their heterosis percentages of up to 28.8 per cent over best parent [7]. Estimates of gca and sca variances will help to detect the amount of additive or dominance and non-additive variances respectively. The average performance of a line in a hybrid combination is indicated by gca while sca is used to identify the best combinations based on the relative performance of the lines involved. An inbred variety cross also will help to determine the gca [24,25].

High sca for drymatter content, maturity, yield, bulb weight and storability were reported in onion hybrids [9,26]. In some crosses both parents contributed high sca for example as in BYG 2207 x Almorah Selection -2 [26]. In aggregate onion hybrids (CS 856-8 x CO-1 and CS 665-51 x CO-1) high degree of gca and sca were observed [27-29]. High gca effects for storage quality were observed in Junagadh White (for TSS and dry matter), Pusa red (marketable quality bulbs) while N53, Bellary Red and Hissar-2 were observed with high gca for yield [30,31].

In the present study, we chose 3 male sterile lines (Lines, L) and 23 restorer lines (Testers, T) with diverse horticultural traits to make crosses in Line x Tester design for precise estimation of gene action and for quantification of degree of divergence among the population for different bulb quality and storage traits and their contribution to the total divergence for net economic yield and keeping quality after harvest.

Materials and Methods

The experiment was conducted twice in three replications for a period of three years. During first year F_1 hybrid seed production was done using three male sterile lines and twenty testers. During second year F_1 hybrid bulb production was done by raising 60 F_1 hybrids, 3 lines, 20 testers and 2 standard checks (total = 85) and the details were presented in table 3. During third year, we studied the post harvest storage quality of harvested F_1 bulbs, parental lines and testers and standard checks.

Experimental material

During 1996-97, the mother bulbs of three male sterile lines (A-lines) and twenty testers (C-lines) were planted in adjacent hills in all possible combinations. The plants of A- line and C-line of adjacent hills were bagged in three-ring muslin cloth bags just before flowering. During flowering all the plants of A-line were tested for pollen sterility and C-line for fertility. Any plant showing fertility in A-line was removed. Similarly in case of C-line, the sterile plants were removed. Everyday the bags were manually shaken for effective pollination. Finally the FI hybrid seed was harvested from A-line (female) plants at the end cropping season. All parents were maintained separately. The F_1 seed of 60 onion hybrids thus harvested in 1997 was used to raise the crop during 1997-98 along with their 23 parents (3 lines and 20 testers) and 2 standard checks to study their performance (Table 3). Total number of 264 treatment combinations in a randomized block design were raised in a net plot size of 0.9 m^2 (3 m x 0.3 m) at a spacing of 15 x 10 cm accomodating 60 plants per plot. During third year, harvested bulbs of these treatment combinations were analysed for their storability at room temperature using various storage structures.

Table 3: Description of the experimental material with symbols and contributed parental traits.

Symbol used for parents and Standard checks	Description of the material	Contributed traits when these parents are involved in "line x tester" breeding cross
Line (L)		
L_1	Pusa Red msms	Less neck diameter, less number of days to maturity, high dry matter and TSS, high vertical diameter and less storage losses
L_2	75 msms	Greater plant height, more number of leaves, high bulb yield, more bulb weight, more horizontal bulb diameter, high dry matter, reduced storage losses.
L_3	(102-1 x 106) msms	Great plant height, greater number of leaves, least number of days to maturity, high bulb yield, average bulb weight, high horizontal bulb diameter, least amount of storage losses.
Tester (T)		
T_1	12-20 P # - 6 # - 3 #	Great horizontal bulb diameter
T_2	44-2#(LR-9A)#-1#	Greatest bulb yield
T_3	113-1-1 ⊗ P_3	Great plant height, greater bulb yield, high TSS and dry matter, less storage loss
T_4	Pusa Ratnar #	Greater horizontal bulb diameter, high TSS, high dry matter, less storage loss
T_5	103 - 3 # ⊗ P_1	Less number of days to maturity, less neck diameter, least storage losses
T_6	New Collection from Daultabad -1 ⊗ P_2	Great plant height, more number of leaves, more bulb yield and bulb weight, greatest horizontal bulb diameter, high dry matter, reduced storage losses
T_7	Hissar II - 2-1-2 ⊗ P_3	Great bulb weight, reduced storage losses
T_8	Red Creole -1 ⊗ P_2	Least number of days to maturity, high bulb yield and bulb weight
T_9	Punjab red Round -1 ⊗ P_1	Greatest vertical diameter, less number of days to maturity
T_{10}	48 - 8 - 5 # - 6 # - 3 #	Great plant height
T_{11}	Arka Kalyan ⊗ P_1	High bulb yield, high dry matter and TSS, reduced storage losses
T_{12}	Agri found Dark Red -1 -2 ⊗ P_2	Greater plant height, high TSS and dry matter, reduced storage losses
T_{13}	45 - 6 - 2 - 1 ⊗ P_1	Less neck diameter, less number of days to maturity ,high bulb yield and bulb weight, less number of days to maturity.
T_{14}	13- 50 Non bolters from October planting # -2 #	Most number of leaves, high horizontal bulb diameter, high bulb yield and bulb weight, less neck diameter, less number of days to maturity, high TSS and dry matter.
T_{15}	400 - 1 - 1 ⊗ P_1	Great plant height, more number of leaves, reduced storage losses
T_{16}	102 - 1 -1 ⊗ P_1	High bulb yield and bulb weight, greater horizontal bulb diameter
T_{17}	14 - 4 ⊗ P_4	Less neck diameter
T_{18}	Bhadurgarh Local -1 -2 ⊗ P_3	High TSS and dry matter, less storage losses
T_{19}	Arka Niketan ⊗ P_1	Less number of days to maturity, less neck diameter
T_{20}	49 - II - 1 # - 1 #	Great plant height,high bulb yield, greater bulb weight, great horizontal and vertical diameters, high TSS, reduced storage losses
Standard Checks (SC)		
SC_1	Pusa Red	
SC_2	Pusa Madhvi	

Experiment location

The experimental plots of the Division of Vegetable Crops at IARI, New Delhi were located at an altitude of 228.6 m above msl with 77° 12' Eastern longitude and 28° 35' Northern latitude. The total rainfall received from end of July to early September during 97 - 98 was 881.1. The mean maximum temperatures (41.3°C) were observed in May while the minimum temperatures were observed in December (8.8°C) and January (5.5°C). The meteorological observations were presented for the cropping period in table 4.

Table 4: Meteorological data during the cropping period in 1997 and 1998.

Name of Month, Year	Temperature (°C)		Relative Humidity(%)		Rainfall (mm)	Sunshine hours
	Min.	Max.	Min.	Max.		
October, 97	17.3	28.8	50.0	82.0	65.8	5.5
November, 97	12.3	24.9	45.0	85.0	17.2	4.5
December, 97	8.8	18.0	66.0	92.0	68.0	1.5
January, 98	5.5	20.5	44.0	56.0	0.0	7.1
February, 98	8.2	24.7	42.0	84.0	29.7	5.6
March, 98	11.6	27.2	44.0	78.0	40.4	7.3
April, 98	19.0	36.1	41.0	67.0	1.4	7.9
May, 98	24.7	41.3	31.6	50.0	7.4	8.2
June, 98	27.1	39.1	48.0	69.0	195.2	5.6
July, 98	27.1	34.3	68.0	82.0	116.3	4.1
August, 98	23.6	40.2	68.0	86.0	158.9	3.3
September, 98	25.9	33.4	70.0	90.0	136.7	5.7
October, 98	19.6	30.7	57.0	88.0	044.1	6.6
Mean (Average)	17.75	30.71	51.89	77.62	67.78	5.61

All the cultural operations starting from nursery to main field preparation, transplanting, fertilization, irrigation, weeding, plant protection were carried out as per the recommendations in oder to raise a successful onion crop.

Observations

The following quantitative and qualitative traits were measured from ten plants selected randomly in each treatment during the experimental period.

Quantitative characters

1. **Plant height:** When the plants were at full growth the height of the plant was measured in centimetres from the base of the plant to the tip of the scallions, tubular hollow green leaves. The mean value from ten plants was taken as the plant height.
2. **Number of leaves per plant**: Total number of green hollow tubular leaves per plant were counted. The mean of ten plants was taken as the number of leaves per plant.
3. **Neck Diameter:** It was measured at peak vegetative growth in centimetres from individual plants with the help of vernier calliper. The mean value from ten plants was taken as the neck diameter.
4. **Plant population per plot:** 60 plants per plot were maintained throughout the experimental period.
5. **Maturity:** It was recorded in days by measuring the number of days taken from the date of transplanting in the main field to the date on which fifty percent neck fall was observed.
6. **Bulb weight:** Ten bulbs were randomly selected and weighed, the mean value is taken the single bulb weight and was expressed in grams.
7. **Bulb yield per plot:** All the bulbs harvested from single plot were weighed and expressed as yield in kilograms per plot.
8. **Bulb diameter:** The vertical and horizontal bulb diameters were measured in centimetres with the help of vernier calliper. The mean value from ten bulbs was taken as the bulb diameter. The shape index was computed by dividing the vertical diameter with the horizontal diameter.

Qualitative characters

- **Total soluble solids (TSS):** The TSS was read on the scale of hand refractometer by placing a drop of extract on the lens screen. The extract was collected by squeezing scales at three locations (middle, interior and outer layers) of each bulb. The mean value of the ten bulbs was taken as TSS of the bulb.
- **Dry matter:** A known amount of onion was dried to a constant weight in a hot air oven at 65°C. The dry matter left behind was expressed as percentage of total solids.
- **Storage loss of bulbs (by percent weight):** Two kilograms of onions were kept in storage for five months from June to the end of October at room temperature for studying storage losses due to rotting, sprouting and shrinkage. Towards the end of October, the final weight of the bulbs was recorded after discarding bulbs spoiled due to sprouting, rotting and shrinkage. The difference was taken as loss in weight and was expressed as percent weight loss of the bulbs during storage.

Statistical analysis

The data collected for all the traits was subjected to analysis of variance (ANOVA) to split the variability into various sources of variation for randomized block design (Table 5). The standard error (SE) of mean (SEm) for genotypes, parents vs crosses as well as for lines vs testers was calculated and respective critical difference (CD) values were deduced at 5 and 1 percent level of error degrees of freedom (df) following standard statistical procedures [32] to understand the significant differences among the hybrids, parents and standard checks. The analysis of covariance (ANCOVA) was performed to know the genotypic and phenotypic co-variances by taking two variables at a time and tested all possible combinations (Table 6).

Table 5: Analysis of variance of the line x tester heterosis breeding experiment using 3 cms lines and 20 testers.

Note: In first column, the values in parenthesis were related to df: Degrees of Freedom; 1: Plant Height (cm); 2 Number of Leaves; 3: Neck Diameter (cm); 4: Days to Maturity; 5: Bulb Yield (kg); 6: Bulb Weight (g); 7: Horizontal Diameter (cm); 8: Vertical Diameter (cm); 9: TSS (%), 10: Dry Matter (%); 11: Storage Loss in Weight (%); NS: Non Significant; *: Significance at 5% Level of Probability and **: Significance at 1% Level of Probability.

Source of Variation (df)	1	2	3	4	5	6	7	8	9	10	11
Replication (2)	1.46	0.05	0.014	5.85	0.026	1.38	0.003	0.003	0.59	1.56	1.73
Genotypes (84)	35.35**	1.17**	0.07**	19.79**	2.92**	823.35**	0.48**	0.23**	3.58**	3.45**	182.16**
Parents (22)	14.14**	1.13**	0.043**	16.99**	2.38**	656.65**	0.68**	0.35**	2.42**	2.22**	55.83
Female (2)	4.12**	1.90**	NS	23.11**	2.11**	586.78**	0.27**	NS	NS	0.17*	39.10
Male (19)	15.83**	0.69**	0.048**	17.00**	2.38**	656.04**	0.63**	0.33**	2.51**	2.11**	53.59
Female vs Male (1)	2.02*	7.88**	NS	NS	2.94**	807.93**	2.49**	1.15**	4.06*	8.52	131.81
Hybrids (59)	42.78**	1.24**	0.072**	21.73**	2.13**	616.94**	0.39**	0.19**	3.92**	3.89**	150.88**
Parents vs Hybrids (1)	35.25**	NS	0.18**	NS	55.23**	16227.19**	1.52**	NS	NS	0.77	4865.61*
Residual (2)	7.60**	0.19*	0.50**	NS	7.94**	3185.13**	0.46**	5.25**	8.12**	10.03**	265.35**
Error (168)	0.35	0.07	0.019	3.18	0.32	2.19	0.03	0.09	0.63	0.85	1.77
CV%	1.00	3.39	9.16	1.60	14.19	2.56	3.28	8.08	7.92	7.90	3.54
CD at 5%	1.33	0.61	0.31	4.05	1.30	3.90	0.39	0.67	1.80	2.07	3.02
CD at 1%	1.75	0.80	0.40	5.32	1.71	5.14	0.51	0.87	2.37	2.73	3.97

Table 6: Analysis of variance for combining ability and estimates of variance components for bulb yield and quality attributes
Note: In first column, the values in parenthesis were related to df: degrees of freedom; 1: Neck Diameter; 2: Days to Maturity; 3: Bulb Yield (kg); 4: Bulb Weight (g), 5: Horizontal Diameter (cm); 6: Vertical Diameter (cm); 7: TSS (%); 8: Dry Matter (%); 9: Storage Loss in Weight (%); The values in parenthesis for the rows corresponding to critical difference (CD) values of lines, testers and hybrids represent CD values at 1% level of significance. σ^2f, and σ^2m represent variance components of female (lines) and male (testers) parents, while σ^2gca and σ^2sca represent CV values of general and specific combining abilities.
NS: Non significant; *: Significance at 5% level of Probability and **: Significance at 1% Level of Probability.

Source of Variation (df)	Mean Sum of Squares								
	1	2	3	4	5	6	7	8	9
Replications (2)	0.01	2.20	0.22	0.18	0.02	0.04	0.14	0.32	0.24
Female (2)	0.10**	45.31**	6.23**	1105.80**	0.25**	NS	10.19**	14.99**	49.08**
Male (19)	0.09**	23.79**	1.85**	641.24**	0.39**	NS	4.51**	2.94**	214.36**
Female x Male (38)	0.10**	19.46**	2.05**	581.43**	0.39**	NS	3.29**	3.78**	124.51**
Error (118)	0.02	2.88	0.33	1.57	0.03	0.61	0.7	0.85	1.26
Lines - at 5% significance	0.03 (0.04)	0.42 (0.55)	0.14 (0.18)	0.25 (0.33)	0.03 (0.04)	0.06 (0.07)	0.22 (0.29)	0.22 (0.29)	0.28 (0.36)
Testers - at 5% significance	0.11 (0.15)	1.30 (1.71)	0.44 (0.58)	0.78 (1.02)	0.11 (0.15)	0.19 (0.26)	0.64 (0.84)	0.69 (0.91)	0.86 (1.13)
Hybrids - at 5% significance	0.14 (0.18)	1.83 (2.40)	0.61 (0.80)	1.08 (1.42)	0.17 (0.22)	0.28 (0.36)	0.92 (1.20)	0.98 (1.31)	1.22 (1.60)
Components of Variance and Combining Ability									
$\boldsymbol{\sigma}^2$f	0.00	0.43	0.07	8.74	0.00	0.00	0.12	0.19	-1.26
$\boldsymbol{\sigma}^2$ m	0.00	0.48	-0.02	6.65	0.00	-0.01	0.14	-0.09	9.98
$\boldsymbol{\sigma}^2$ gca	0.001	0.44	0.06	8.47	0.002	0.003	0.12	0.15	0.21
$\boldsymbol{\sigma}^2$ sca	0.015**	0.53**	0.58**	193.29**	0.122**	-0.003	0.86**	0.98**	41.10**

ANCOVA

Source	df	MP	Expected MP
Replications	(r-1)	MP_{r12}	
Genotypes	(g-1)	MP_{g12}	$\boldsymbol{\sigma}^2e_{12} + \sigma^2g_{12}$
Error	(r-1)(g-1)	MP_{e12}	$\boldsymbol{\sigma}^2e_{12}$

Table a

Where,

r and g are the number of replications and genotypes respectively and "σ^2e_{12}, σ^2g_{12}" are the error and genotypic covariance of characters X_1 and X_2 respectively.

Genotypic covariance = $\sigma^2g_{12} = (MP_{g12}-MP_{e12})/ r$

Phenotypic covariance = $\sigma^2P_{12} = \sigma^2e_{12} + \sigma^2g_{12}$

Heterosis

Heterosis percentage was calculated over the better parent, top parent and standard check for all quantitative characters, as follows:

- Heterosis over better parent (BP) = $[(F_1 - BP)/BP]x100$
- Heterosis over top parent (TP) = $[(F_1-TP)/TP]x100$
- Heterosis over standard check (SC) = $[(F_1-SC)/SC]x100$

For testing the significance of heterosis, SE and CD were calculated as described below.

SE = $\sqrt{(2 \times V_E)}/r$, where V_E is the error mean sum of squares of RBD and r is number of replications.

CD = SE x 1.414 't' value at 5 and 1 percent level of error df

The ANOVA and ANCOVA analysis tables were provided (Table 5, 6).

Combining ability

The variation among the hybrids for all the characters was partitioned into general (gca) and specific (sca) combining ability components by the standard model given below following the standard procedures [33].

$$Y_{ijk}=\mu + f_i + m_j + S_{ij} + e_{ijk}$$

Where, Y_{ijk} is the value of any character measured of the cross "i x j" in the k^{th} replication, μ is the over all mean, f_i is the gca effects of i^{th} parent (female), m_j is the gca effects of j^{th} parent (male), S_{ij} is the specific effects of the progeny of i x j and e_{ijk} is the random error effect associated with ijk observation.

Based on this model, the following ANOVA was set up.

Source of variation	Degrees of freedom (df)	Mean sum of squares (MS)	Expectation of MS
Replications	(r-1)		
Females	(f-1)	M_1	$\sigma^2e + r\sigma^2_{fm} + rm\sigma^2_f$
Males	(m-1)	M_2	$\sigma^2e + r\sigma^2_{fm} + rf\sigma^2_m$
Females x Males	(f-1)(m-1)	M_3	$\sigma^2e + r\sigma^2_{fm}$
Error	(r-1)(fm-1)	M_4	σ^2e

Table b

Where,

f, m, r represent the number of females, males and replications, respectively; σ^2e represent the genetic variance among individuals from the same mating; σ^2_f is the progeny variance arising from the differences among the female parents; σ^2_m is the progeny variance arising from the differences among the male parents; σ^2_{fm} is the progeny variance arising from the interaction of the contribution of the female and male parents; while MS is the mean sum of squares.

Solving the simultaneous equations arising as a result of equating the expectation of MS to the actual MS gave values for the estimates of variance components. They were formulated as follows:

$\sigma^2_f = (M_1 - M_3)/rm$

$\sigma^2_m = (M_2 - M_3)/rf$

$\sigma^2_{fm} = (M_3 - M_4)/r$

σ^2_f g.c.a. $= \sigma^2_m + \sigma^2_f$

σ^2_f s.c.a. $= [(M_3 - M_4)/r] \times \sigma^2_{fm}$

Test of significance of estimates of variances: 'F' test was utilized to test the significance of variance estimates.

To test $\sigma^2_f = 0$, $F = M_1/M_2$ at df (M_1) and df (M_2) respectively.

To test $\sigma^2_m = 0$, $F = M_2/M_3$ at df (M_2) and df (M_3) respectively.

To test $\sigma^2_{gca} = 0$, $F = [d\,(m-1)(f-1)] = M_*/M_3$

Where,

$* = (mM_2 + fM_1)/m+f$

$d = [(mM_2 + fM_1)^2\,(m-1)(f-1)]/[(f-1)(m^2M_2)^2 + (m-1)(f^2M_1)^2]$

iv) To test $\sigma^2_{sca} = 0$, $F = M_3/M_4$ at df (M_3) and df (M_4) respectively.

Estimation of general and specific combining ability effects

The gca (g_i) and sca (S_{ij}) effects were obtained from the two way table of females vs males in which each figure was a total over replications.

$ij = (x....)/rfm$; where, $x.... = f>_{i=1}\ m>_{j=1}\ r>_{k=1} X_{ijk}$

$f_i = [(x_{i...})/rm] - [(x....)/rfm]$; where, $x_i.... = m>_{j=1}\ r>_{k=1} X_{ijk}$

$m_{ij} = [(x_j....)/rf] - [(x....)/rfm]$; where, $x_j.... = f>_{i=1}\ r>_{k=1} X_{ijk}$

$S_{ij} = [(x_{ij}....)/r] - [(xi)/rm] - [(xj)/rf] + [(x....)/rfm]$; where, $x_{ij} = r>_{k=1} X_{ijk}$

Standard error of general and specific combining ability effects and critical difference

The standard error of effects was calculated as square root of the variances of effects. The variances of the various effects were calculated as follows;

gca effects of females

$Var.(f_i) = [\sigma^2_e\,(f-1)] / rfm$

$Var.(f_i - f_j) = [2\,\sigma^2_e] / rm$-----$j \neq i$

gca effects of males

$Var.(m_i) = [\sigma^2_e\,(m-1)] / rfm$

$Var.(m_i - m_j) = [2\,\sigma^2_e] / rf$ -----$j \neq i$

sca effects of hybrids

gca effects of females

$Var.(S_{ij}) = [\sigma^2_e\,(f-1)\,(m-1)] / rfm$-----$j \neq k$

$Var.(S_{ij} - S_{jk}) = [2\,\sigma^2_e(m-1)] / rm$-----$i \neq k$

The critical differences were calculated by multiplying the SE$\sqrt{2}$ with t value at 5 and 1% level of probability for error degrees of freedom.

Multivariate analysis by D^2 statistic

Mahalanobis D^2 - Statistic is used for assessing the genetic divergence between populations [34]. The generalized distance between any two populations is defined as

$\Delta^2 = (\lambda^{ij})\,\delta_i\,\delta_j$

Where, (λ^{ij}) is the reciprocal matrix to the common dispersion matrix and δ_i is the difference between the mean values of the two populations for the i^{th} character. The quantity is estimated by D^2-statistic [35] as $D^2 = (S^{ij})\ \delta_i\ \delta_j$, where, ($S^{ij}$) is the sample estimate of (λ^{ij}) and δ_i of δ_j.

Since the formula for computation requires the inversion of the matrix of fourteenth order, transformation of the original correlated, unstandardized character means to standardized uncorrelated variables was done to simplify the computational procedure. The transformation was effected by Pivotal condensation method [36].

The various group constellations in the sample study are determined by Tocher's method as described [36]. After the formulation of the groups on the basis of D^2 values, the intergroup divergence was obtained by calculating the average D^2 between any two groups i.e. D^2 of each member of one constellation and every member of the other constellation. All these computational calculations were carried out in SPAR1 programme on PC486 computer.

Results and Discussion

The analysis of variance (ANOVA) for RBD for eleven characters studied gave 1) significant differences for male tester parents, mean sum of squares (MSS) due to treatment means, contribution of parents and hybrids towards variation among genotypes while 2) non-significant variation was observed between female ms parents, bulb and neck diameters, number of leaves, days to maturity in specific treatment combinations of parents and hybrids (Table 5). The analysis of covariance data as well as the best performing parent, F_1 hybrid and the standard check (SC) and their values, number of heterotic hybrids with significant heterosis over better parent (BP), top parent (TP) and standard check (SC) as well as F_1 hybrids exhibiting highest heterosis percentages over the BP, TP and the SC are presented along with their images (Table 6, 7; Figures 1a to 4c). The best hybrids with their per se performance in all the traits tested were presented in table 8. The mean values obtained from averages (samples and replication) of respective lines (female parents), testers (male parents) (Table 9) and hybrids (Tables 9a to 9c) were presented as well as heterosis percentages of best five F_1 hybrids over the mid parent (MP) are also presented (Table 10).

Figure 1: a: Bulbs of Pusa Red msms (L_1), Arka Kalyan $\otimes$ P_1 (T_{11}) and their F_1 hybrid. b: Bulbs of Pusa Red msms (L_1), Agri found Dark Red-1-2 $\otimes$ P_2 (T_{12}) and their F_1 hybrid. c: Bulbs of Pusa Red msms (L_1), 45-6-2-1 $\otimes$ P_1 (T_{13}) and their F_1 hybrid.

Figure 2: a: Bulbs of Pusa Red msms (L_1), 13-50 Non-bolters from October planting # -2 # (T_{14}) and their F_1 hybrid. b: Bulbs of 75 msms (L_2), New collection from Daultabad-1 ⊗ P_2 (T_6) and their F_1 hybrid. c: Bulbs of 75 msms (L_2), Hissar II-2-1-2 ⊗ P_3 (T_7) and their F_1 hybrid.

Figure 3: a: Bulbs of 75 msms (L_2), Red Creole –1 ⊗ P_2 (T_8) and their F_1 hybrid. b: Bulbs of 75 msms (L_2), Arka Kalyan ⊗ P_1 (T_{11}) and their F_1 hybrid. c: Bulbs of 75 msms (L_2), 49-II-1#-1# (T_{20}) and their F_1 hybrid.

Figure 4: a: Bulbs of (102-1 x106) msms (L_3), Red Creole –1 ⊗ P_2 (T_8) and their F_1 hybrid. b: Bulbs of (102-1 x106) msms (L_3), Arka Kalyan ⊗ P_1 (T_{11}) and their F_1 hybrid. c: Bulbs of (102-1 x106) msms (L_3), Bhadurgarh Local –1-2 ⊗ P_3 (T18) and their F_1 hybrid.

Table 7: Comparison of onion F_1 hybrids over better parent, top parent and standard check for yield attributes.

Note: The data columns represent characters from 1: Plant height (cm); 2: Leaf number; 3: Neck Diameter (cm); 4: Maturity in Days, 5: Bulb Yield (kg); 6: Bulb Weight (g); 7: Horizontal Diameter (cm); 8: Vertical Diameter (cm); 9: TSS (%); 10: Dry Matter (%); 11: Storage Loss in Weight (%).

* and ** represents number of hybrids with significance at 5% and 1% level of probability respectively.

Where TP, BP and SC represent top parent, better parent and standard check respectively.

Item	1	2	3	4	5	6	7	8	9	10	11
Best F_1 hybrid (Value)	L_2xT_{20} (67.58)	L_3xT_{14} (9.53)	L_1xT_{13} (1.53)	L_3xT_8 (112.67)	L_2xT_6 (6.54)	L_2xT_6 (106.33)	L_2xT_6 (6.02)	L_1xT_9 (4.23)	L_1xT_{12} (12.67)	L_1xT_{12} (14.91)	L_3xT_{11} (25.42)
Best Top parent (Value)	T_3 (61.54)	L_1 (9.53)	T_8 (1.67)	T_{10} (118.33)	T_2 (4.78)	T_2 (79.67)	L_1 (5.94)	T_5 (4.43)	T_9 (11.93)	L_2 (12.72)	L_1 (38.33)
Best Standard Check (Value)	SC_1 (60.76)	SC_1 (8.17)	SC_1 (1.83)	SC_2 (121)	SC_2 (3.06)	SC_2 (65)	SC_2 (5.21)	SC_2 (3.85)	SC_1 (12.33)	SC_1 (13.52)	SC_1 (28.87)
Total number of heterotic F_1s											
Over BP	29	9	25	33	36	36	14	9	10	8	68
-at 5% -at 1%	20* 20**	9* 9**	12* 12**	8* 4**	36* 36**	36* 36**	13* 13**	8* 8**	7* 6**	6* 6**	66 63
Over TP	15	-	5	2	17	17	2	-	2	8	49
Over SC	16	9	19	19	57	40	32	17	1	4	13
-at 5% -at 1%	12* 8**	9* 9**	15* 15**	3* 2**	57* 57**	34* 33**	30* 30**	17* 17**	1* 1**	4* 4**	6* 6**
Best F_1 hybrid											
Over BP (Heterosis%)	L_2xT_{20} (13.26)F	L_3xT_{14} (19.67)F	L_1xT_{13} (-20.69)F	L_1xT_{13} (7.35)M	L_2xT_6 (85.80)F	L_2xT_6 (81.23)F	L_3xT_{14} (11.33)M	L_1xT_6 (19.06)F	L_1xT_{12} (19.97)F	L_1xT_{12} (19.97)F	L_3xT_{11} (-44.58)M
Over TP (Heterosis%)	L_2xT_{20} (9.81)	- -	L_1xT_{13} (-8.38)	L_1xT_{13} (-2.81)	L_2xT_6 (36.82)	L_2xT_6 (34.04)	L_2xT_6 (1.35)	- -	L_1xT_{12} (6.20)	L_1xT_{12} (17.22)	L_3xT_{11} (-33.68)
Over SC (Heterosis%)	L_2xT_6 (6.30)	L_3xT_{14} (16.65)	L_1xT_{13} (-16.39)	L_3xT_8 (-6.88)	L_2xT_6 (131.73)	L_2xT_6 (63.58)	L_2xT_6 (15.55)	L_1xT_9 (9.87)	L_1xT_{12} (2.76)	L_1xT_{12} (10.28)	L_3xT_{11} (-11.34)

Table 8: Hybrids possesing sca effects in the desired direction for bulb yield, yield components and keeping quality.

Character/Trait	Hybrids with Desired sca effects	Per se performance of the hybrid	Gca effects of parents involved
Plant height (cm)	L_3 x T_{12} L_1 x T_6 L_2 x T_{20}	63.24 67.10 67.58	LxL LxH HxM
Number of leaves/plant	L_3 x T_{14} L_2 x T_{15}	9.53 9.40	LxH HxL
Neck Diameter (cm)	L_1 x T_{13} L_3 x T_5 L_2 x T_{14}	1.53 1.60 1.80	HxL LxL LxL
Days to maturity	L_1 x T_{13} L_3 x T_9 L_1 x T_2	115.00 118.00 119.67	LxH HxL LxL
Bulb yield (Kg/plot)	L_2 xT_6 L_2 x T_8 L_1xT_{11}	6.54 6.16 5.18	HxL LxL LxL
Bulb weight (g)	L_2 xT_8 L_2 x T_{20} L_3 x T_{18}	102.67 92.97 90.33	HxL HxM LxL
Horizontal bulb diameter (cm)	L_2 x T_{20} L_1 x T_{17} L_2 x T_6	5.57 5.65 6.02	HxL LxL LxM
Vertical Bulb diameter (cm)	L_2 x T_{20} L_2 x T_6 L_1 x T_9	4.03 4.17 4.23	LxL LxL LxL
Total soluble solids, TSS (%)	L_1 x T_{12} L_2 x T_{14} L_3 x T_7	12.67 10.87 11.07	LxM HxL LxL
Dry matter (%)	L_1 x T_{12} L_2 x T_6 L_1 x T_3	14.91 14.68 13.41	HxL HxM HxL
Storage loss in weight (%)	L_1 x T_{18} L_1 x T_3 L_1 x T_7	35.65 29.75 35.33	LxL LxL LxL

Note: In column 2, L_1 to L_3 are lines and T_1 to T_{20} are testers for the respective hybrids while in column 4, the letter L, H and M represent Low, high and Medium respectively.

Table 9: Average performance of the parents for eleven characters studied during the experimental period.

Parents	1	2	3	4	5	6	7	8	9	10	11
L_1	57.41	9.53	1.93	119	4.76	79.33	5.94	4.13	10.13	12.43	38.33
L_2	59.67	8.50	1.83	124.33	3.52	58.67	5.54	4.13	10.73	12.72	40.33
L_3	57.91	7.97	1.90	120.33	3.16	52.67	5.35	3.75	11.13	12.24	45.33
T_1	59.28	8.20	2.17	122	3.60	60.00	5.92	3.85	8.00	9.25	53.17
T_2	55.51	7.60	1.73	121.67	4.78	79.67	5.05	3.33	10.47	11.99	45.00
T_3	61.54	7.50	1.80	120.67	3.74	62.33	5.57	3.70	10.00	12.23	41.67
T_4	59.05	7.00	1.80	122	2.03	33.83	4.66	3.50	10.47	11.33	43.50
T_5	59.15	7.27	1.70	123.33	4.20	70.00	5.62	4.43	10.33	11.12	48.83

T_6	56.76	8.00	1.80	119	2.29	38.17	4.82	3.84	9.53	12.60	40.50
T_7	55.99	7.40	1.90	122.67	4.63	77.17	4.94	3.60	10.20	11.82	41.50
T_8	54.91	7.33	1.67	122	3.39	56.50	5.37	3.92	10.87	11.40	40.58
T_9	54.63	8.27	1.77	121.33	3.28	54.67	4.92	3.50	11.93	12.68	43.00
T_{10}	54.95	7.40	1.93	118.33	3.96	66.00	5.29	4.00	8.60	10.44	53.17
T_{11}	57.29	7.53	1.83	122.67	2.18	36.33	4.28	3.19	11.27	12.58	47.82
T_{12}	56.80	8.07	2.07	122.67	2.63	43.83	5.64	3.83	9.40	10.99	44.65
T_{13}	60.23	7.07	1.93	123.33	2.90	48.33	4.81	3.74	9.80	11.25	44.31
T_{14}	55.41	7.40	1.77	119.67	2.71	45.20	4.79	3.28	9.67	10.56	44.43
T_{15}	61.23	7.40	1.87	119.67	1.92	32.00	4.79	3.10	10.00	11.34	44.25
T_{16}	61.29	7.80	1.83	126.33	3.40	56.66	4.11	3.14	9.87	11.73	43.64
T_{17}	60.31	7.47	1.70	121.67	2.28	38.00	4.93	3.42	9.80	11.47	39.67
T_{18}	57.57	7.47	1.90	123	4.24	70.67	5.25	3.60	8.73	10.55	53.33
T_{19}	56.92	8.10	1.80	128.33	3.56	59.33	5.37	3.84	10.67	11.91	47.69
T_{20}	57.52	9.00	2.00	119.33	2.31	38.50	4.80	3.55	9.33	11.16	48.00

Note: The first column represent parents where L1 to L3 are male sterile lines and T1 to T20 are testers used for onion hybridization program. Data columns represent averages of samples and replications of 23 parental genotypes for 1: Plant Height; 2: Leaf Number; 3: Neck Diameter; 4: Maturity in Days; 5: Bulb Yield (kg); 6: Bulb Weight (g); 7: Horizontal Diameter (cm); 8: Vertical Diameter (cm); 9: TSS (%); 10: Dry Matter (%); 11: Storage Loss in Weight (%).

Table 9a: Average performance of the F_1 hybrids from L_1 (Pusa Red msms) for eleven characters studied during the experimental period.

Note: The first column represent parents of F_1 hybrids from cms line L_1 with testers T_1 to T_{20} used for onion hybridization program. Data columns represent averages of samples and replications of 20 hybrid progenies for characters 1: Plant Height; 2: Leaf Number; 3: Neck Diameter; 4: Maturity in Days; 5: Bulb Yield (kg), 6: Bulb Weight (g); 7: Horizontal Diameter (cm); 8: Vertical Diameter (cm); 9: TSS (%); 10: Dry Matter (%); 11: Storage Loss in Weight (%).

F_1 onion Hybrids	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
L_1xT_1	62.18	8.40	1.93	123.33	4.00	66.67	5.11	3.83	9.07	11.36	36.17
L_1xT_2	54.13	7.33	1.83	119.67	3.64	60.67	4.87	3.43	9.60	11.70	38.75
L_1xT_3	57.35	7.10	1.77	121.33	4.12	65.33	4.98	3.53	11.47	13.41	29.75
L_1xT_4	59.25	7.33	1.93	121.00	4.12	65.33	5.50	3.84	11.60	14.03	28.50
L_1xT_5	61.56	8.03	1.97	127.33	3.40	56.67	5.07	3.47	10.53	12.25	30.17
L_1xT_6	67.10	9.47	1.97	124.00	5.14	85.67	4.89	3.34	10.01	11.25	34.50
L_1xT_7	61.57	7.27	1.97	121.33	4.40	73.33	5.09	3.67	9.80	12.47	35.33
L_1xT_8	55.89	7.70	1.57	123.33	3.12	52.00	5.65	3.93	10.00	11.53	30.17
L_1xT_9	56.90	7.60	1.80	127.67	4.03	67.33	5.28	4.23	10.80	12.45	28.44
L_1xT_{10}	56.53	8.13	2.00	121.67	4.28	71.33	5.77	4.07	7.80	10.17	51.40
L_1xT_{11}	58.50	7.53	1.93	127.00	5.18	86.33	5.55	3.80	11.33	13.11	27.60
L_1xT_{12}	56.32	6.20	1.67	120.33	3.32	55.33	4.95	3.48	12.67	14.91	28.50
L_1xT_{13}	55.83	7.00	1.53	115.00	5.48	91.33	5.11	3.57	9.73	11.04	38.11
L_1xT_{14}	59.65	7.73	2.00	119.00	5.54	92.33	5.50	4.09	7.20	9.23	52.68
L_1xT_{15}	59.71	7.47	2.03	127.67	3.34	55.67	5.17	3.75	9.87	12.29	37.42
L_1xT_{16}	59.90	7.63	2.07	123.67	5.39	95.33	5.66	3.84	9.33	11.19	38.12
L_1xT_{17}	56.64	7.87	1.90	122.33	3.88	64.67	5.65	3.89	10.53	11.04	30.67
L_1xT_{18}	61.70	7.80	2.00	123.33	3.55	59.17	5.48	3.89	10.07	11.75	35.65
L_1xT_{19}	56.91	7.47	1.63	121.67	4.64	76.33	5.46	3.76	10.13	11.37	36.67
L_1xT_{20}	57.49	7.80	1.90	120.33	2.64	44.00	4.09	3.12	11.47	12.77	28.83

Table 9b: Average performance of the F_1 hybrids from L_2 (75 msms) for eleven characters studied during the experimental period.

Note: The first column represent parents of F_1 hybrids from cms line L_2 with testers T_1 to T_{20} used for onion hybridization program. Data columns represent averages of samples and replications of 20 hybrid progenies for characters 1: Plant Height; 2: Leaf Number; 3: Neck Diameter; 4: Maturity in Days; 5: Bulb yield (kg); 6: Bulb weight (g); 7: Horizontal Diameter (cm); 8: Vertical Diameter (cm); 9: TSS (%); 10: Dry Matter (%); 11: Storage Loss in Weight (%).

F_1 onion Hybrids	1	2	3	4	5	6	7	8	9	10	11
L_2xT_1	58.05	7.70	1.97	122.00	5.34	89.00	5.54	3.84	10.47	12.58	33.92
L_2xT_2	61.85	8.07	2.07	122.00	4.00	66.67	5.37	3.54	10.53	12.90	30.14
L_2xT_3	62.96	7.40	1.83	120.67	3.44	57.33	4.93	3.23	8.87	10.38	46.49
L_2xT_4	58.90	8.83	2.00	121.67	4.40	73.33	5.64	3.83	10.27	11.91	30.67
L_2xT_5	61.39	7.73	1.97	127.67	4.67	77.83	5.20	3.32	9.87	10.95	38.50
L_2xT_6	64.59	9.00	2.03	129.33	6.54	106.33	6.02	4.17	12.33	14.68	27.44
L_2xT_7	59.12	8.07	1.97	123.67	5.92	98.67	5.63	3.95	9.47	11.47	39.75
L_2xT_8	61.51	7.80	2.10	122.67	6.16	102.67	5.51	3.91	10.20	12.43	32.25
L_2xT_9	53.15	7.73	1.70	120.33	4.00	66.67	5.15	3.59	10.80	11.82	28.20
L_2xT_{10}	62.78	8.00	1.97	123.00	4.70	77.67	5.35	3.56	9.73	10.61	36.42
L_2xT_{11}	57.83	7.60	1.73	121.00	3.22	53.67	4.91	3.35	11.13	12.22	27.11
L_2xT_{12}	56.67	8.60	2.10	122.33	3.64	60.67	5.16	3.65	10.67	11.37	32.17
L_2xT_{13}	60.48	8.00	2.07	123.67	5.34	88.33	5.61	4.03	10.47	12.22	33.50
L_2xT_{14}	54.99	7.07	1.80	118.67	4.72	78.67	5.24	3.55	10.87	12.15	34.17
L_2xT_{15}	61.73	9.40	2.00	122.00	5.00	83.00	5.21	3.98	10.40	12.41	32.46
L_2xT_{16}	60.46	7.60	1.87	128.67	5.70	95.00	5.51	3.74	9.80	11.66	42.11
L_2xT_{17}	54.02	7.33	1.80	122.33	3.44	57.33	4.67	3.60	10.80	12.31	30.33
L_2xT_{18}	61.95	8.73	2.20	119.67	4.70	78.33	5.62	3.97	9.93	11.56	46.34
L_2xT_{19}	60.50	7.93	1.77	121.33	3.88	64.67	5.49	3.67	10.20	12.21	33.00
L_2xT_{20}	67.58	7.87	2.00	121.67	5.36	92.97	5.57	4.03	10.33	12.23	29.08

Table 9c: Average performance of the F_1 hybrids from L_3 (10^2-1 x 10^6 msms) for eleven characters studied during the experimental period.

Note: The first column represent parents of F_1 hybrids from cms line L_3 with testers T_1 to T_{20} used for onion hybridization program. Data columns represent averages of samples and replications of 20 hybrid progenies for characters 1: Plant Height; 2: Leaf Number; 3: Neck Diameter; 4: Maturity in Days; 5: Bulb yield (kg); 6: Bulb Weight (g); 7: Horizontal Diameter (cm); 8: Vertical Diameter (cm); 9: TSS (%); 10: Dry Matter (%); 11: Storage Loss in Weight (%).

F_1 onion Hybrids	1	2	3	4	5	6	7	8	9	10	11
L_3xT_1	60.97	7.93	2.17	123.33	4.67	82.33	5.69	3.40	9.60	11.07	46.35
L_3xT_2	54.68	7.80	1.87	122.67	4.24	70.67	5.64	3.92	9.07	11.06	44.45
L_3xT_3	58.31	7.73	1.77	120.67	3.44	57.33	5.07	3.75	9.27	11.00	39.82
L_3xT_4	60.41	7.40	1.93	121.33	4.70	78.00	5.59	3.83	10.87	12.08	30.11
L_3xT_5	58.75	7.07	1.60	121.33	4.96	82.33	5.26	3.91	11.07	12.11	28.93
L_3xT_6	52.57	8.73	2.13	120.33	4.24	71.33	5.51	3.69	10.73	11.36	27.08
L_3xT_7	61.60	8.30	2.13	123.33	4.63	77.00	5.23	3.29	11.07	12.27	26.50
L_3xT_8	60.60	7.40	1.83	122.67	2.88	47.67	4.81	3.53	7.42	9.04	49.03
L_3xT_9	50.68	7.47	1.93	118.00	3.76	62.67	5.46	3.60	9.00	10.21	41.82
L_3xT_{10}	58.36	8.47	1.93	122.33	4.64	77.33	5.56	4.11	9.27	11.57	39.12
L_3xT_{11}	58.16	7.67	1.93	120.00	4.28	71.33	5.61	3.56	11.20	12.98	25.42
L_3xT_{12}	63.24	7.77	1.93	122.33	4.64	77.33	5.16	3.59	9.47	10.81	44.08
L_3xT_{13}	52.86	7.00	1.97	117.67	3.90	65.00	5.26	3.61	10.07	11.78	28.02
L_3xT_{14}	60.26	9.53	2.13	122.33	4.90	81.67	5.96	3.67	9.27	11.21	33.42
L_3xT_{15}	62.62	7.00	2.00	122.67	3.62	60.33	4.97	3.53	9.07	10.06	38.67
L_3xT_{16}	56.29	7.47	1.87	120.67	3.97	65.33	5.05	3.77	9.60	11.70	40.33

L_3xT_{17}	47.00	6.80	1.57	119.00	3.29	54.67	4.61	3.21	10.47	11.78	28.37
L_3xT_{18}	59.72	7.93	1.97	121.00	5.42	90.33	5.52	3.95	7.67	9.20	51.79
L_3xT_{19}	52.49	7.20	1.77	119.67	3.76	62.00	4.61	3.51	9.20	10.42	29.42
L_3xT_{20}	58.56	8.07	2.07	121.33	4.60	76.67	4.95	3.53	9.27	10.39	27.51
The data columns in tables 9, 11, 12, 13 were averaged and grand mean values of 11 traits and SEm were provided below	60.76 58.78	8.17 7.90	1.83 2.03	122.00 121.00	2.29 3.06	65.00 41.33	4.78 5.21	3.79 3.85	12.33 10.47	13.52 11.93	28.67 33.14
Grand mean	58.53	7.81	1.89	122.05	4.01	67.42	5.24	3.69	10.04	11.67	37.60
SEm+/-	0.48	0.22	0.11	0.15	0.47	1.41	0.14	0.24	0.65	0.75	1.09

Table 10: The best onion F_1 hybrids identified with desired quality attributes based on mid- parental(MP) values

Character/Trait	Top five hybrids with their heterosis(%) in parenthesis
Bulb yield	Hybrids of L_1 with testers T_{11}(49.28) > T_{14}(48.24) > T_{13}(43.08) > T_{16}(32.16) > T_4(21.35) > T_{19}(11.54). Hybrids of L_2 with testers T_6(125.13)> T_{20}(83.98) > T_{15}(83.82) > T_8(78.29) > T_{13}(66.36). Hybrids of L_3 with testers T_4(81.12) > T_{20}(68.29) > T_{14}(66.95) > T_{11}(60.30) > T_6(55.60)
Bulb weight	Hybrids of L_1 with testers T_{11}(49.28) > T_{14}(48.21) > T_6(45.82) > T_{13}(43.08) > T_{16}(40.20) Hybrids of L_2 with testers T_6(119.60) > T_{20}(90.70) > T_{15}(83.08) > T_8(78.29) > T_{16}(65.32). Hybrids of L_3 with testers T_4(80.35) > T_{20}(67.58) > T_{14}(66.78) > T_{11}(60.29) > T_{12}(60.27).
Days to Maturity	Hybrids of L_1 with T_{13}(-5.55) > T_{19}(-1.76) > T_2(-0.60) > T_{14}(-0.30). Hybrids of L_2 with testers T_{19}(-4.30) > T_{18}(-3.52) > T_{14}(-2.98) > T_9(-2.22) > T_{11}(-2.20). Hybrids of L_3 with testers T_{19}(-4.08) > T_{13}(-3.73) > T_9(-2.56) > T_{16}(-2.35) > T_{17}(-1.80)
Neck diameter	Hybrids of L_1 with testers T_3(-20.69) >T_{12}(-16.67) >T_8(-12.96) > T_{19}(-12.50) > T_1(-5.69). Hybrids of L2 with testers T_9(-5.56) > T_{11}(-5.45) > T_{19}(-2.75) > T_1(-1.67). Hybrids of L_3 with testers T_{17}(-12.96)>T_5(-11.11) > T_3&T_{19}(-4.50 > T_{12}(-2.52).
Horizontal bulb diameter	Hybrids of L_1 with testers T_{16}(12.67) > T_{11}(8.68) > T_{17}(4.05)>T_4(3.77)>T_{10}(2.79) Hybrids of L2 with testers T_6(16.25) > T_{16}(14.21) > T_4(10.62) > T_{13}(8.47) > T_{20}(7.84). Hybrids of L_3 with testers T_{14}(17.55) > T_{11}(16.40) > T_4(11.58) > T_6(8.39) > T_{16}(6.69).
Vertical bulb diameter	Hybrids of L_1 with testers T_9(10.84) > T_{13}(10.53) > T_{11}(3.83) > T_{14}&T_{15}(3.69) > T_{16}(3.00). Hybrids of L2 with testers T_{15}(10.15) > T_{20}(4.95) > T_6(4.77)> T_7(4.16) > T_{16}(2.94). Hybrids of L3 with testers T_2(10.63) > T_{16}(9.28) > T_{18}(7.52) > T_{10}(5.93) > T_4(5.51).
Total soluble solids(TSS)	Hybrids of L_1 with testers T_{12}(29.69) > T_{20}(17.81) > T_3(13.91) > T_4(12.62) > T_{11}(5.92) Hybrids of L_2 with testers T_6(13.82)> T_1(11.74)> T_{14}(6.54)*> T_{12}(5.96)>T_{15}(5.19). Hybrids of L_3 with testers T_6(3.87)>T_7(3.75) > T_5(3.11).
Dry Matter	Hybrids of L_1 with testers T_{12}(27.33) > T_4(18.10) > T_3(8.76) >T20(8.27) > T_{11}(4.84). Hybrids of L2 with testers T_6(15.58)>T_1(14.52)> T_{14}(4.37)> T_{15}(3.16) > T_8(3.05). Hybrids of L_3 with testers T_{11}(4.32)> T_1(3.02)> T_5(3.71)> T_4(2.50)> T_{10}(2.03).
Storage losses in weight	Hybrids of L_1 with testers T_{11}(-35.93) > T_{20}(-33.21) > T_{12}(-31.31) > T_5(-30.77) > T_4(-30.34). Hybrids of L_2 with testers T_{11}(-38.49) > T_{20}(-34.16) > T_9(-32.31)> T_8(-32.10) > T_2(-29.36). Hybrids of L_3 with testers T_{11}(-41.86) > T_6(-41.47) > T_{20}(-41.04)> T_7(-38.97) > T_5(-38.55).

Note: All hybrids are significant at 1 % level of significance except those marked with * are at 5% probability.
The symbols L_1 to L_3 are lines and T_1 to T_{20} are testers for the respective hybrids.

The parents providing the highest number of leaves per plant, plant height, bulb weight, bulb yield, total soluble solids (TSS), dry matter, bulb diameter (both vertical and horizontal) and showing early maturity, less neck diameter, less storage losses were considered as better parents. Therefore, positive heterosis in the former and negative heterosis in the latter case over desired parents were considered for comparison. Because there is no genetical method for testing the significance of superiority of the F_1 hybrids over the top parent, the values were taken as such for comparison. We confined our discussion to the parameters such as bulb maturity, bulb diameters, bulb yield, total soluble solids and percent loss of bulbs because these are correlated directly to post harvest storage losses in F_1 hybrids of onion derived from its cytoplasmic-genic male sterile lines.

In the present study, the analysis of variance for combining ability indicated significant differences among parents (i.e. females and males) and female x male for all the characters studied except for vertical bulb diameter. A comparison of parents vs crosses exhibited significant differences for all characters studied except for days to maturity and vertical bulb diameter, indicating that the F_1's were almost on par with their parents. However, the replication mean sum of squares for all the characters were found to be non-significant. The combining ability for variance components reveals that relative magnitude on non-additive component, for all the characters except vertical bulb diameter, was greater than additive component indicating predominance of non-additive gene action governing those characters in certain hybrid combinations (Table 5, 6). These results were in conformity with previous observations of predominance of sca affects in F_1 hybrids of onion [3].

Over all, lines possessed greater gca variance than testers for the characters bulb yield, bulb weight and dry matter where as lines were good combiners for these three traits compared to testers. However tester had good gca variance for many traits like plant height, number of leaves per plant, days to maturity, TSS and storage loss of weight suggesting that testers are good combiners for these five traits (Table 5, 6).

Effect of parental genome on bulb yield and its earliness in F_1 hybrids

Onion germplasm evaluations in the past revealed its major contribution of bulb yield towards genetic divergence [3,37] indicating the influence of parental genome. The heterosis observed in onion F_1's is due to favourable genes accumulated for a trait either by dominance of both parents, allelic interaction or over domiance and non-allelic interaction or epistasis at heterozygous gene loci of one of their parents. These genetic factors of additive (gca) and non-additive (sca) gene action were discussed below for these two traits.

BULB YIELD: is a dependant variable on complex growth parameters besides the major contribution of parental genotype. In the present study, the heterosis perecentages for yield ranged from 3.41 to 85.80 which were in line with the previous reports of 24.9 to 83.8 per cent in onion F_1 hybrids using cms lines [3,38,39]. The bulb yield of superior SC (75msms) and TP (T_2) were 3.06 and 4.78 respectively. Out of 36 superior F_1 hybrids over BP, the hybrid, L_2 x T_6 gave maximum heterosis (85.80%) for bulb yield over BP (36.82%) and SC (113.73%). This hybrid combination was followed by L_2 x T_8, L_2 x T_{16} and L_3 x T_{14}.

Only two lines (L_1 and L_2) and seven testers (T_3, T_7, T_{12}, T_{13}, T_{14}, T_{16},T_{17}) resulted in significant gca affects for yield. The significance is identified both within and among parental lines for gca of this trait. Out of these parents, only one line (L_2) and four testers (T_7, T_{13}, T_{14} and T_{16}) were observed with significantly high positive gi effects and were identified as good general combiners for bulb yield. The tester T_{14} showed the highest gca of 0.72 closely followed by T_{16} (0.69) and hence these testers were resulted in production of good quality traits when crossed with all three female cgms lines. Thirteen hybrids expressed significantly positive sca effects with a greatest S_{ij} effects for bulb yield in hybrid L_2 x T_8 (1.74) followed by L_2 x T_6 (1.43). There were hybrids with S_{ij} values lower than 0.75, but proved potential in heterotic yields which include in the decreasing order based on their S_{ij} value were L_3 x T_{12} (0.88) > L_1 x T_{13} (0.83) > L_1 x T_{19} (0.80) and L_2xT_{20} (0.80).

The two testers (T_{14} and T_{16}) out of the four best with greater gca resulted in heterotic hybrids with the promising ms line 2 (75 msms) as expected due to additive gene action. The cms line L_2 proved as the best female parent for bulb yield when crossed with best testers as well as the testers with low significant g_i values like T_6, T_8, T_{11} and T_{20} which is unexpected improvement in bulb yield due to non-additive gene action during hybridization indicating the dominance of L_2 masking the effects of the recessive genes of this trait that were present in these inferior testers. Similarly, F_1 hybrids with additive genetic variance for bulb yield were resulted from crossing L_3 or L_2 as females with four promising testers, while non-additive genetic actions of these lines for combining ability were observed with other testers having less promising gi effects (negative gca values).

In the present investigation, only six hybrids (L_2 x T_2; L_2 x T_3; L_2 x T_{11}; L_3 x T_2; L_3 x T_3; L_3 x T_8) out of 60 hybrids produced were a failure due to their high negative heterosis for yield and its attributes (Tables 9a to 9c). The critical and favourable conditions of growth were another reason for unexpected yield of some onion hybrids (Table 4), though genetic potential of at least one parent is the main reason for high yields. The parents with negative gca were highly ineffective if chosen for hybridisation purposes. For example, the promising line L_2 was ineffective in boosting the yield of the less promising testers like T_2, T_3, and T_{11} as well as L_3 and T_8 combination. In rare incidences like in combinations of L_3 x T_{11} and L_2 x T_{11} producing quality bulb yields even though 1) tester T_{11} recorded with very low bulb yield (2.18) compared to other lines (Table 9, Figures 3b and 4b) and 2) cgms line L_3 was observed with negative g_i effects for bulb yield.

The heterosis percenatge for tester 11 was positive with L_3 (34.44 Female), negative with L_2 (-8.52 Female) and L_1 (8.83 Female) for bulb yield which were resulted from the trait specific dominant gene contribution of respective female parents in the specified hybrid combinations. Therefore, the dominant gene from female parent will lead the positive directional heterosis for yield when crossed with inferior testers with recessive allele at a specific gene locus for this trait on the genome. In our research study, tester 11 "Arka Kalyan ⊗ P_1 " was rare tester observed with good comnbining ability to produce heterotic F_1 hybrids using cytoplasmic-genic male sterility. This tester explained the major contributing genetic factors of female fertile (male sterile) cms lines towards bulb yield irrespective of the gi and sij effects observed in twenty polygenetically diverse male fertile tester parents used in our study. Our findings on heterosis observed were confirmed with those observed when Arka Kalyan tester crossed with Telagi red line at IIHR, Bangalore in India [38].

Our results were in line with the previous findings of high gca for yield in onion cv. Pusa white flat, Selection 102-1 and Punjab Selection [3,8]. Previously, the herotic values of F_1s produced using cms lines ranged in percentages from -26 to 192 and 31 to 367 over BP and OPV respectively as well as bulbs of F_1s were highly uniform (100%) with high yield (2 - 27% over OPV) and marketability since 1950s [40]. The F_1s from a cross combination of Valencia with Ebenzera and Oriental x Odonoletnij Sibirkij were highly heterotic for earliness and productive yield [41]. There were so many heterotic F_1 hybrid seed, produced using ms lines ,when direct seeded resulted in earliness, greater bulb weight with good bulb characters, only a few were presented from our study here with images with related data (Figures 1a to 4c; Table 8 and 10). The favourable growth conditions providing optimum temperature (18-31°F), rainfall (67.78mm), relative humidity (52 - 78%) and sunshine hours (5.61) boosted the production of F_1 hybrids proving the key contributing factor of environment towards gene expression (Table 4). These results were in line with some of such previously released hybrids as listed in Table 2 that included Young Early Extra (27-41.8% higher yield), and Rannii 1 (matures in 110 days) [42-44].

BULB WEIGHT: It is one of the major yield contributing factors just like vegetative characters viz., number of leaves, plant height, number of root and root growth that have direct control over "source-sink" relationship for final productivity. Fifty percent of our F_1 onion hybrids were promising with positive heterobeltiosis (3.92 to 81.23%) and standard heterosis (0.51 to 63.58%) while only 17 hybrids were observed best over top parent. The F_1 hybrid L_2 x T_6 recorded maximum heterosis over BP (81.23%), SC (63.58%) and TP (34.04%). The F_1 hybrids in the order of their superioty with heterosis for average bulb weight were L_2 x T_6>L_2 x T_8> L_2 x T_7>L_2 xT_{16} indicating ms line 2 is very good in contributing this trait to the F_1s. These results were supported by onion heterotic F_1 produced in 1992 [45] and 1988 [3]. The reasons for superiority in hybrid produced from crosses with line 2 (75 msms) and testers T_6, T_7, T_{15}, and T_{20} were high gca effects for plant height that lead to the development of leaves, bulb weight and size and ultimately yield (Tables 6, 7, 9, 9a, 9b and 9c). The testers T_6, T_7, T_{16}, and T_{14} were observed with significant gca effects for bulb weight. Fifty three hybrid combinations were observed with siginifcant sca effects among which L_2 x T_8 > L_2 x T_{20} > L_3 x T_{18} > L_3 x T_{12} > L_2 x T_6 > L_3 x T_5 > L_1 x T_{13} > L_1 xT_{17} exhibited high sca effects over BP.

EARLINESS: Days to maturity determines earliness in harvesting matured onion bulbs to have bulbs of desired moisture level for long term storage and long distance transport as well as to minimise losses during storage. This trait was observed with extreme significance with negative heterosis ranging from -0.58 to -7.35 per cent indicating F_1 hybrids produced with earliness ([7,43,44], Tables 8 and 9). The trait "days to maturity" was contributed to majority of hybrids produced with ms line 3 (102-1x106) msms. The superior F_1 hybrid identified was L_3 x T_8 with maturity days of 113 days (actual value is 112.67 days) with negative heterois percentages of -6.37 over BP, -4.78 over TP and -6.88 over SC (Table 7, Figure 4a). The hybrids L_1 x T_{13}, L_3 x T_{19} and L_1 x T_{19} observed superior after L_3 x T_8. The lines L_1 and L_3 and testers T_8, T_{13} and T_{19} were good combiners for earliness (Table 9). Further, the combinations L_1 x T_{13}, L_3 x T_5, L_3 x T_6 and L_3 x T_9 were also observed with high negative sca effects for days to maturity and found promising. Thus, ms line "Pusa Red" followed by "(102-1 x106)" msms were unique for earliness, quality and quantity of bulbs. Few other testers T_1, T_{10}, T_{12}, T_{18} and T_{20} were observed with earliness when crossed with this line indicating that at least one parent must be a good combiner with negative heterosis for this trait to result in early maturity of onion bulbs. After critical analysis of our germplasm, the possible reason for observed earliness in F_1 onion hyrbids evaluated in our study might be attributed to both line and tester parents used for heterosis breeding and their rooted origins of ancestral collections of original germplasm from temperate zones of India and the United States of America.

Genetic divergence

Large genetic diversity observed in onion F_1 hybrids [16] and majority of this diversity is contributed by polygenic traits but not

due to their geographic region of origin (Tables 1, 2, 3; [16]). The selection was mostly done based on phenotypic traits of plant growth, yield contributing parental traits like early maturity, bulb size, bulb weight, and storability (Tables 8 and 10).

The inter cluster distance ranged from 145.41 (Cluster II and V) to 400.28 (Cluster II and IV) while intra-cluster distance ranged from 0 (Cluster II) to 153.35 (cluster I). A maximum number of genotypes were grouped in cluster IV with minimum intra-cluster distance (T_4, T_6, T_{11}, T_{13}, T_{14}, T_{15}, T_{16} and T_{17}) and these were collected from Delhi, Haryana and Bangalore regions. Cluster I had five genotypes (T_1, T_{10}, T_{12}, T_{18} and T_{20}) collected from Delhi, Nasik and Haryana regions while cluster III also had five genotypes (L_3, T_2, T_7, T_8 and T_9) collected from Delhi, Haryana, Ludhiana and USA. Only one genotype was grouped in cluster I (L_1) collected from Delhi while cluster V had four genotypes (L_2, T_3, T_5 and T_{19}) collected from USA, Delhi and Bangalore. Here, L_1 (Pusa Red msms) is unique and stood at the top in contributing better yield and quality. In Cluster III, cms line L_3 was associated closely with testers T_2, T_7, T_8 and T_9 which may provided the reasons for observed earliness as expected in hybrids obtained from these testers. Further in cluster V, L_2 is closely associated with T_3, T_5 and T_{19} that indicated the above average performance of hybrids produced from these testers (Tables 7, 8, 9, 9a, 9b, 9c and 10).

The percent contribution of qualitative parameters for divergence in the decreasing order were: Bulb weight (37.50), > Storage loss of weight (29.61), > Plant height (24.90), > Horizontal bulb diameter (3.56), >Number of leaves per plant (0.198), > TSS (1.20), > dry matter (0.80), > days to maturity (0.40). The neck diameter and vertical bulb diameter did not contribute towards genotypic divergence [16]. The genetic divergence data provided information that the lines and testers are on par with each for the traits analysed and highly prolific in producing heterotic hybrids indicating a need for researching genotypes in each single cluster based on phenotypic traits correlating biochemical traits governing quality during storage and later correlating with identified molecular markers for these agro-morphological traits for quick selection of best hybrids and for eliminating less promising homozygous lines in the field evaluation stage.

The free and frequent exchange of genetic material among the farmers and breeders in the country makes it very difficult to maintain the real identity of genotype. Some lines might were not provided with actual location of the origin, though a state or region name is provided to find out the exact cause for observed genetic diversity. Moreover, the breeding progenies incorporate genes from varied sources, thus losing the basic geographical identify of the forces other than geographical origin, such as exchange of genetic stocks, genetic drift, spontaneous variation, natural and artificial selection that are responsible for genetic diversity. Thus, a correct and dependable record of germplasm with their origin with source for collection is always necessary for a breeder to rescue the potential breeding lines without losing their dominant genes confering yield and quality.

Influence of parents (male sterile lines and testers) on bulb size and quality F_1 hybrids

BULB SIZE of F_1 hybrids is measured by vertical and horizontal diameters of the bulb and it was observed significantly superior over BP, TP and SC in few crosses out of 60 hybrids studied (Tables 6 and 7). The positive heterobeltiosis for horizontal bulb diameter ranged from 0.66 to 11.33 per cent. The F_1 produced from L_2 x T_6 recorded maximum standard heterosis followed by L_3 x T_{14} and L_3 x T_1 for horizontal bulb diameter. The positive heterobeltiosis for vertical bulb diameter ranged from 0.36 to 19.06 per cent. The hybrid L_1 x T_9 gave the highest vertical diameter of the bulb (4.23 cm) with heterobeltiosis of 2.42 per cent and standard heterosis of 9.87 per cent. The parents (L_1 and L_2) with high gca contributed increases in bulb diameters of F_1 due to expressed high sca values in hybrids. The hybrid L_2 x T_{20} was found to be the best for bulb diameter (both vertical and horizontal).

High gca effects for neck diameter was recorded in T_{19}, T_{17}, T_{14} and T_6 while testers T_4, T_{10} and T_{14} for horizontal bulb diameter and T_{18} for vertical bulb diameter. These testers were considered good combiners for the traits of bulb size in F_1 hybrids. The tight bulbs thick with scales and vertical diameter might be useful to make onion fries just like french fries where as circular shaped onion rings are most common with bulbs possessing best horizontal bulb diameter. The cross combinations viz., L_1 x T_{13}, L_3 x T_5, L_2 x T_{14} and L_3 x T_{17} are observed with good negative sca effects for neck diameter. These traits of neck diameter and bulb size in terms of diameter are in favour of quality of bulbs produced by these F_1 hybrids as well as for food processing industry.

BULB QUALITY: is studied in terms of TSS and dry matter content for nutritional point of view. Only 7 hybrids identified superior for TSS over BP while only one hybrid over TP and SC. The hybrid L_1 x T_{12} had highest TSS (12.67%) with an observed heterosis of 25, 6.2 and 2.76 percentages over BP, TP and SC respectively. This was followed by L_1 x T_4 and L_1 x T_{20} indicating that cms line Pusa Red was a contributor of this trait due to its high gca values of these promising hybrids (Tables 6, 7, 9, 9a, 9b, and

9c). But there were F_1 crosses with good TSS content even from the parents with low gca effects and this might be due to non-additive gene action as supported from previous investigations in onion [3,46]. The other F_1 hybrids that were observed with high TSS and drymatter with high sca include L_1 x T_{12}, L_1 x T_3, L_2 x T_{14}, L_2 x T_6, L_1 x T_{18}. Superior onion inbreds and populations were developed using recurrent - selection strategies that increased the frequency of desirable alleles with additive effects. Further, in the past when onion populations maintained by open pollination were observed with phenotypic variation for size, shape, pungency, soluble solid contents, skin and flesh color, maturity, storage ability and bulbing response under different day lengths. The enzymatic puruvic acid concentration, a measure of pungency and SSC were good estimates that can determine additive gene action, might contributed postharvest storage quality in selecting some of our best F_1 hybrids. A variation in long day and short day onions in storage quality clearly indicates that molecular and biochemical analysis of these F_1 hybrids and their parents might reveal clues about unique fragments confering identified better storage attributes of the germplasm evaluated and the cellular level reasons for the observed hybrid vigor [7,28,46,47].

Dry matter was observed with high heterotic effects with a positive heterosis range of 0.25 to 19.97 per cent and standard heterosis of 3.77 to 10.28 per cent. The hybrids with maximum heterosis in the decreasing order were L_1 x T_{12} > L_2 x T_6 > L_1 x T_4. Previously hybrids with high dry matter (13.73%) were developed with high heterosis having marketable bulbs [7,9,13,48]. For this trait, at least one parent with high dry matter resulted in F_1 with high dry matter. The ms lines Pusa Red msms and 75msms were observed with high gca whose contribution might have resulted in enhanced dry matter contents in all the hybrids produced using these two lines. The testers observed with high gca for dry matter and TSS included T_4, T_6, T_{11}, T_{12}.

To study the superiority of the hybrids, we subjected data to statistical analysis using midparental (MP) values and the best five hybrids were presented with bulb quality attributes (Table 10). It was revealed from the results that three male sterile female parents viz., Pusa Red msms, 75 msms and the (102-1x106)msms contributed greater percentage of negative heterosis for neck diameter and storage losses in bulb weight. Only two testers in each line viz., T_{10} and T_{10} when crossed with Pusa Red msms, T_3 and T_{16} when crossed with 75msms and T_{18} and T_{17} when crossed with (102-1x106) msms respectively resulted in poor keeping quality (Tables 9a to 9c). Further, the number of hybrids produced due to gca effects, with reduced neck diameter were very less (5 to 8) from these three lines. This explains predominance of the non-additive effects due to sca that resulted in majority (54 out of 60) of hybrids with non-negative values for neck diameter. Therefore, it is advantageous to use cytoplasmically male sterile female parents with nuclear genes for fertility restoration in onion to boost production with excellent bulb quality. Our studies also indicated importance of future research on biochemical anlysis of these hybrids and parents to understand the potential contributing factors for minimizing storage losses in bulbs when stored at ambient room temperatures and relative humidity.

Over all, the hybrids L_1 x T_{12} followed by L_1 x T_3, L_2 x T_{14}, L_2 x T_6 and L_1 x T_{18} were best combinations for TSS and dry matter with high sca effects and due to high gca effects of atleast one parent based their superiority over SC, BP, TP, and MP. The observed heterosis in F_1 hybrids for yield and storage quality is mainly due to the high frequency of dominant allele at nuclear fertility restoration locus in onion populations used as male testers to cross female ms lines with dominant S cytoplasm. The heterozygous loci in male testers were the true contributors of beneficial yield and desirable quality attributes observed in the present study by eliminating the less desirable gene loci from female lines. Therefore, heterosis is counted again to find out the superiority of F_1 over the mean values of the sterile female line and testers (Table 10). A test cross of these superior F_1 hybrids might be helpful to know the gene loci conferring superior traits of female lines as we observed all three lines producing good quality F_1 for more than one trait. Previous researchers explained this as an increase in additive gene action of desirable alleles by open population of inbreds, if they possess a recessive allele that allows seed propagation of sterile lines with S cytoplasm [47]. Thus, molecular marker analysis of parents and hybrids is a need in the presented indigenous long day onion germplasm of India not only to conserve the true onion lines with potential genes, but also to enhance the desirable quality attributes of the onion bulbs.

Influence of storage time, atmosphere and packing structures on keeping quality of onion bulbs

Stage of harvesting is the prime factor controlling keeping quality of onions. Lack of storage facilities is another main factor contributing crop losses particularly during short day onion harvesting seasons. The weight losses were minimised when the leaves were completely bent and half dried while harvesting onions. About 35 per cent bulb losses during storage were recorded [49].

From our investigation, significant negative heterosis for bulb weight loss was observed in 56 hybrids out of 60 studied (Tables 9a to 9c). The F_1 hybrid L_3 x T_{11} observed with lowest percentage of storage losses (25.42) with high percentages of negative heterosis over BP, TP and SC after five months of storage. The L_2 x T_{11}, L_3 x T_{20} and L_1 x T_{11} were the next best hybrids with better storage quality and with reduced losses due to rotting, sprouting or shrinkage. The parents L_2, L_1, T_6, T_7, T_8, T_9, T_{11} and T_{12} were having better storage qualities and these traits were contributed to F_1 hybrids. It was also observed that hybrids with high TSS and low neck diameter resulted in high keeping quality in onion hybrids and in parents during our investigation. Our observations were supported [3] superior to the recorded losses (49.5 to 51.23%) in storage after 8 months [23].

The parents and hybrids were identified with good storage quality upto 5 months under ambient conditions of 28 - 32°C matching with earlier research reports in onion lines viz., AC 50, 139, 141, TA 258, 264, 377, 278, 383 and 387. We observed an increase in losses during initial months due to physiological weight loss followed by bulb rotting due to slow rise in temperatures with advance in season and later by sprouting due to vernalization caused by low temperature [21,50]. Our findings were in line with the total cumulative losses observed in weight and losses due to rotting and sprouting that accounted to 14 to 37.5 per cent in these cultivars, very less when compared to more than 60 percent losses observed in commercial check varieties, Granex 429, Superex and Texas Early Grano 502 [18,20].

Our results of observed maximum keeping quality in hybrids produced from T_{11} [Arka Kalyan $\otimes P_1$] were in contrary to the previously reported maximum losses in Arka Kalyan, but were supported by the results of the hybrids produced from three cms lines with minimum losses as reported in Pusa Red and Punjab Red Round [19]. The storage quality of parents and hybrids was so much diverse supporting the findings of some cultivars (Seroli 1) with recorded minimum losses while others with maximum (VL 1) losses in storage [21] and while few cultivar (N-2-4-1) identified as promising for a period of more than five months storage as well [23].

The major reason for storage losses were due to rotting associated with increased temperatures with RH (>95%). The bulbs were very good at 65- 75 per cent RH. The losses during first month of storage were attributed to physiological weight loss or rotting while those in later months to sprouting. Onions with tight neck and base like in cv. Texas Grano 1025Y contributes reduced storage losses by avoiding water loss through neck and basal ends [22].

From our data, the tester T_{11} [Arka kalyan$\otimes P_1$] was observed with highest positive gca effects for characters like TSS, dry matter and highest negative gca for storage losses in weight. The testers T_{11}, T_{12}, T_4 and T_6 were found to be good combiners for TSS as well as for dry matter while testers T_{11}, T_{20}, T_6, T_4 and T_{17} were found to be good combiners for reduced storage loss in weight. Previous findings reported that onion cv. Selection 126 was observed with high gca for processing (dehydration ability) and storage quality [3]. The cross combinations L_1 x T_{18}, L_1 x T_3 and L_3 x T_{18} were found to be the best crosses for less storage losses in weight due to their high sca affects. Surprisingly, the parents in L_1 x T_{18}, were observed with low gca effects for this trait indicating a non-additive gene action for improved storage quality trait in this hybrid.

It has been observed that dry matter, TSS and pungency are positively correlated and show moderate to high heritability, which allows simultaneous improvement through selection for these traits. Onion-processing industries require less pungent onions with long shelf life, high TSS and high dry matter contents as well as cultivars with single-centre bulbs for onion ring industry. We observed a lot of genetic variation in bulb size, some hybrids with extreme vertical diameter can be selected and improved to produce bulbs for onion fries. These long onion fries also help reducing packing space unlike onion rings occupying lot of space. Due to considerable variation in onion bulb colour, and its genetics, it is possible to do recurrent or pureline selection of inbreds to produce F_1 with preferred color and pungency to benefit health, nutrition and industry [51].

Influence of packing material on storage quality of onion bulbs

In good olden days, onions were stored and transported in gunny bags made out of jute. These bags are having pore space for aeration and gaseous exchange to avoid spoilage due to rotting. The onions were dried on the floor spread with these gunny bags to dry bulbs immediately after harvest. To date, this is the valuable practice in South Indian conditions and in regions where there is no danger of wetness or moisture during storage or marketing of the harvested and dried bulbs. Further, the damage (bruised or broken) caused during harvesting and packing might contribute losses in marketable quality of onion. This error is unavoidable (Figure 8) if the packing material is not soft enough (cardboard or foam) to protect the damage during transport, but can be controlled at harvest time by managing the depth and width of harvest by prior monitoring of the lines and rows in a field plot.

For onion storage, now plastic netted bags with facility for air circulation around the bulbs has improved shelf life in temperate regions where there is possibility of weight loss due to rotting

and sprouting due to low temperatures. In temperate regions, usually sprouting occurs during the times of spring (15 January to 15 February) which is associated with black mold and eventual rotting. These observed variations in the storage quality of dried and harvested bulbs of certain cultivars might be attributed to physiological changes of those matured bulbs leading to biochemical conversions of stored energy compounds (sugars). During storage these compounds in the form of hormones and energy were supplied to the stem, and these initiated sprouting under normal conditions of storage (Diurnal Temperature Day: 40 - 50°F/night: 20 - 30°F; 0% RH). This was observed in both temperate and tropical regions and was more commonly observed in purple onions (Figure 7).

In our investigation, we stored our bulbs from various treatments (parents, F_1 hybrids) in the iron cabinets inside small gunny bags at our IARI vegetable research farm's produce storage building. To compare the storage quality, storage treatments also included plain ground, racks for direct storage without using gunny bags. The differences between fresh weight and dry weight measured the storage loss in quality. The rotten or damaged onions were discarded after taking observation on percent loss in storage at three periodical intervals during five months time of storage. During these three intervals, we observed a gradual variation in storage quality as influenced by physiological and biochemical factors conferring bulb quality in storage. From our storage studies and the data obtained from field (Tables 9, 9a, 9b and 9c), by critical study of means of parents and hybrids revealed that most the hybrid bulb yields exhibited the tendency towards tallness. The cross combinations with L_2 contributed towards vegetative growth in field and eventually resulted in greater bulb weight, bulb size and yield where cms line 1 and 3 are mostly contributed towards quality with all the 20 testers studied in storage. The cross combinations $L_1 \times T_{13}$, $L_3 \times T_{11}$ produced hybrids with reduced neck diameter and less storage losses respectively where "Pusa Red msms" and "(102-1x106) msms" contributed positively for better postharvest storage quality. The hybrids produced with three female ms lines possessing comparatively higher plant height, yield per plot, bulb weight, bulb diameter, dry matter, lesser neck diameter, less number of days to maturity and lesser storage losses were presented as images (Figure 1a to 4c). These results first gave us information on the genetic potential that contributed reduced storage losses by gca and sca effects (Tables 8, 9, 9a, 9b, 9c and 10).

Later after observation of genetic divergence data and its correlation with the morphological features gave us lot of information on the quality and the contribution of environment. Twenty three parental genotypes were grouped into five clusters based on D^2 values. All the clusters had genotypes collected from Delhi besides a unique cluster II with only one cms line Pusa Red msms that contributed yield and quality in most of the hybrids produced. While earliness and storage quality were only contributed by cms lines 2 and 3 which were grouped in two separate clusters respectively with testers obtained from Bangalore, Haryana, Ludhiana. Surprisingly, both of these clusters were having gene pool from USA. The reasons for earliness and quality in these clusters could be due to genetic drift and selection under different environments after introduction for quality traits. The hybrids produced though did not show much difference in yield phenotypically but did expressed their diversity in quality which was mainly due to environment interaction, a character acquired due to change in growing climate and temperature. Further, the diversity analysis also revealed a secret that white onions (T_{18}) were having better storage quality compared to purple, yellow or brown onions which were matched with our phenotypic observations. The reason for high quality of $L_3 \times T_{18}$ is not only due to ms line "(102-1x106) msms" but also due to the tester "Bhadurgarh Local-1-2 $\otimes P_3$".

The red (purple) onions were highly prone to lot of damage due to sprouting when compared to brown (white) onions. The red onions that were in round shape were comparatively with tight neck compared to oval shape (Figure 7). The oval shape red onions were highly prone to rotting. The yellow onions were observed with loose scales in orientation around the bulb not providing sufficient insulation to the edible bulb inside, that might have lead to spoilage by fungi or bacteria besides rotting. An analysis of these microbia might be useful to know the name of the fungi or bacteria causing the damage as well as the right post-harvest stage that is most prone to the microbial damage. The reason for shelf life variation by bulb color is attributed to genotype potential. The white color onions with brown dried scales outside (Figures 5, 6, 7, 8) were highly potential in providing tightness not only at the two ends of base and neck, but also to insulate the entire bulb by the outer dried scales through-out its entire surface and circumference. These tight scales are actually not letting the inside bulb moisture to evaporate due to outside temperature variations in the storage environment and serving as water proof material to avoid moisture entering from outside.

Several molecular techniques were in place today to assess the genetic diversity for maximum hybrid vigor and to identify superior hybrids. The inbred lines 356-2 (flat, dark red, and matures in 95 days) and 378 M (oval, dark red, and matures in 90 days) were identified with high genetic distance and maximum performance based on RAPD (Random Amplified Polymorphic DNA) marker

Figure 5: Heterosis (%) over better parent for bulb yield.

Figure 6: Heterosis (%) over better parent for percent loss in weight after 5 months of storage.

Figure 7: Variation in storage quality due to tight stem base, color, shape, neck diameter, thickness of dried scales in onions marketed today.

Figure 8: Packing material of today in temperate regions for storage and distribution of onions.

data that eliminated lot of investment in field evaluation and to reduce the time for selection of parents [17].

Study of onion genotypes and hybrids, that were developed as well as those cultivars and hybrids that were released commercially for massive production in India or worldwide, would help in revealing the biochemical basis for long term storage and finding the total genetic variability present in the population. These biochemicals can lead to identify the genes coding for phenotypic traits of long term storage to use in marker assisted molecular breeding in onion. The phenotypic markers identified for long shelf life are 1) round shape, 2) tightness of the bulb scales, neck and base, 3) color of the bulb - cultivars with pure white color bulb covered with brown dried scales (or any identified bulb color with tight scales), and this trait offers resistance from pathogens and 4) thin outer scales - this trait is visible specifically in white (brown) onions while it was absent in purple and yellow onions. The thin scaly leaves are dried faster, silky, water proof and help to insulate the bulb with fleshy edible scales at a faster rate requiring less drying time. The thick scales were taking long time for drying after harvest compared to thin scaled tight bulbs of specific genotypes.

Our research findings in storage quality were supported as the quality of onion cultivars is determined by bulb colour (anthocyanin and flavonoid content), firmness, number of scales, number of growing points, neck thickness, total soluble solids (TSS), pungency and antioxidants [51]. Therefore, it would be beneficial to study the biochemicals contributing bulb color, firm ness and thickness of scales (both inner and outer scales). Analysis of pungency and antioxidants would help in improving industrial value for temperate regions required with low pungency onions. As of today, the pungency of onions marketed globally was very less when compared to 1990s, though the bulb size has increased from pin head size multiplier onions to cricket ball size Pusa Red hybrids. The predominance of non-additive gene action for most of the yield attributing traits in our onion F_1s will provide an opportunity to exploit the frangibility of heterosis breeding program in onion.

Further, development of such onion cultivars with tight scales will also help 1) to adopt and resume our organic farming practices in onion production, 2) to support mechanisation or automation in post harvest processing for industrial use 3) to reduce load and time during the process of dehydration in industry 4) to resist damage during transit.

Over all, the testers T_6, T_7, T_8, T_9, T_{11}, T_{12}, T_{13}, T_{14}, T_{18} and T_{20} were observed for selection with good general combining ability though the promising three cms lines vary with their gca contribution based on the potential tester with which they were crossed.

Conclusion and Future Thirst

Out of 60 hybrids, 54 hybrids were observed with good yield and quality traits. The best 3 to five hybrids listed (Tables 8 and 10) will be useful for further postharvest biochemical evaluation for storage quality to study the pathways that were genetically controlled. Some of these hybrids were released commercially for bulb yield with proven quality in India by onion breeders. Onion hybrids identified from our research study might be useful for processing industry. The investigations on storage qualities of F_1 bulbs will be useful for future generations of research in post

harvest technology and onion production. The lines that produced F_1 hybrids with high TSS with less storage losses and better bulb size and yield are good combiners of heritable variation and hence can be used in commercial breeding programs of onion. Future studies directed towards correlation of genetic similarities with morpho-agronomic, biochemical and molecular markers might be necessary to chose the best crosses from our study. These potential lines or testers as well as F_1 hybrids can used in tissue culture as well to conserve germplasm, and develop protocols for vegetative means of propagation from stem base aseptically for uniform bulbs while avoiding massive investment on breeding programs in onion. Such vegetative propagules can be helpful in massive onion production using aquaponics. These methods not only increase marketable onions with best quality free from rotting or sprouting, but also reduce the seed cost and unit purchase price of onion bulbs. Because tissue culture methods help to produce genetic-cytoplasmic male sterile parental plants in sufficient numbers for commercial hyrbid seed production.

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