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GENETIC VARIABILITY, TRAIT LINKAGE AND GENETIC DIVERSITY IN BROCCOLI

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ABSTRACT

The experiment confirmed that high altitudes of the Eastern Ghats in Andhra Pradesh are suitable for cultivation of broccoli during *Rabi* season. The analysis of variance revealed that presence of significant genetic variation among the 9 cultivars for all the characters studied. Moderate level of PCV and GCV were recorded for plant height, days taken to 50% head initiation, head weight, head diameter and yield per hectare. High heritability combined with high genetic advance was recorded for all the traits studied, except leaf length and width indicated that these characters are command by additive gene action. So, straight selection may be followed for the improvement of broccoli for these traits. Trait association of nine broccoli cultivars indicate that yield/ hectare recorded the high positive association with head weight. The genotypes Palam Kanchan and Palam Samradhi were highly diverse genotypes. Hybridization between these two would give superior variants in segregating generations that would give better performance in this region.

Key words : PCA, High Altitude zone, Broccoli, Genetic variability, Genetic diversity.

Introduction

Broccoli is a winter season vegetable crop, mainly grown at an optimum temperature range between 16^0 and 20^0C **(**Karistsapol *et al*., 2013). The USA is the most predominant producer of broccoli in all over the world. The cultivation of broccoli is now in advance popularity among Indian farmers for the last couple of years apparently due to increasing awareness of its high nutrition values and more scope for expansion of crop due to availability of suitable climatic conditions.

The broccoli has high nutritional value when compared to other Brassicaceae vegetables like cabbage and cauliflower in protein (3.6%), fat (0.3%), carbohydrate (5.9%), vitamin A (9000 I.U.), calcium (2-16%) and iron (684 ppm). Broccoli also contains a chemical known as Indole-3-Carbinol, which is believed to have anti-cancerous properties (Choudhury, 2005). It is fairly rich in carotene, ascorbic acid and also contains better quantities of thiamin, riboflavin, niacin and iron.

Into India, broccoli was introduced much later among the cole crops in 1990s. Since then its cultivation has remained in hills and plains of North and some parts of East India. Low temperatures sufficient for the growth and development of broccoli happen during the winter season in Eastern Ghats in Andhra Pradesh. The climatic conditions of the high elevation and tribal zone of Andhra Pradesh, India with the maximum temperature range 17 to 35ºC, while the minimum temperature fluctuates from 3 to 24ºC is more favorable for the cultivation of high-value Brassicaceae vegetable like broccoli. But, no effort has been made on the assessment of genetic variability of broccoli cultivars under the high altitude and tribal areas of Andhra Pradesh. Hence, as a first and foremost step for identification and developing varieties suitable for these regions, it is necessary to evaluate the performance of broccoli in these regions during winter/ rabi season.

The performance of any crop or variety mainly depends upon its genetic makeup. In addition, the performance of the cultivar of the crop depends upon climatic conditions of the region under which the crop or cultivar grown. As a result, genotypes which perform well in one zone might not perform well in other zone of varying climatic. So, it is highly necessary to collect and asses all the available genotypes to select desirable high

yielding genotypes or cultivar for a particular agro-climatic condition. Considering the nutritional value, increasing awareness on health and increasing demand for broccoli, research on this crop is highly necessary to find out the suitability of various genotypes for this particular region.

Investigations on varietal evaluation and genetic diversity were made previously by some workers (Habib *et al*., 2013; Vivek *et al*., 2014; Nandhini *et al*., 2019) in broccoli in other parts of the country but not in the Eastern hills of South-India. Therefore, the present investigation was taken up to test the suitability of prevailing weather conditions in this region for broccoli cultivation, to assess various genetic parameters, to determine the direct and indirect effects of constituent characters on yield and to study genetic diversity among nine broccoli genotypes in Eastern Ghats of Andhra Pradesh.

Materials and Methods

An experiment was conducted at open field to estimate the genetic variability, heritability and genetic advance for different characters and also to estimate characters association in nine diverse broccoli genotypes. The experiment was carried out in the randomized block design in three replications during *Rabi* 2017-18, 2018-19 and 2019-20 at Horticultural Research Station, Chintapalli, Andhra Pradesh, India. The experimental location falls under Agro-climatic zone of High elevated and Tribal Zone with the average yearly rainfall of more than 1300 mm and is situated at an altitude of 933 m MSL (Siva *et al*., 2020). The ecological condition of the experimental location is 17^0.13' N latitude and 84^0.33' E longitudes. The soil of the experimental field was alluvial with good drainage facilities.

The mean values were used to statistical analysis of data for each character as per the method is given by Panse and Shukhatme (1985).

Genotypic Co-efficient Variation (GCV) and Phenotypic Co-efficient of Variation (PCV) were estimated as per Burton (1952) and Broad sense Heritability (h^2_{bs}) was estimated as stated by Hanson *et al.* (1956), whereas Genetic Advance as per cent Mean (GAM) was estimated as given by Johnson *et al*. (1955).

$$\text{Genotypic Variation}\left(\sigma_g^2\right)=\frac{\text{MSS due to genotypes}-\text{MSS due to G}\times\text{E}-\text{MSS due to error}}{\text{r}\times\text{E}}$$

$$\text{GCV}\left(\%\right)=\frac{\sigma_g\times 100}{\text{Mean}}$$

$$\text{Phenotypic Variation}\left(\sigma_p^2\right)=\sigma_g^2+\frac{\text{MSS due to G}\times\text{E}}{\text{r}\times\text{E}}+\frac{\text{MSS due to error}}{\text{r}}$$

$$\text{PCV}\left(\%\right)=\frac{\sigma_p\times 100}{\text{Mean}}$$

$$h^2_{bs}\left(\%\right)=\frac{\sigma_g^2\times 100}{\sigma_p^2}$$

$$\text{GAM}=\frac{\sigma_p\times h^2_{bs}\times 2.06\times 100}{\text{Mean}}$$

Here, r = number of replications and E = number of years.

Genotypic correlation coefficients and phenotypic correlation coefficients were calculated as per the formulae proposed by Johnson *et al*. (1955). The significance of the phenotypic correlation coefficients and genotypic correlation coefficients was calculated as per formula of Snedecor and Cochran (1967).

The principle components (PCs) were calculated by using the method suggested by Gower (1966). The mean genotype values were applied to the hierarchical cluster analysis. The software employed were R version 4.0.5 for ANOVA and Tukey's Honest Significant Difference test and MINITAB v 19 for PCA and Cluster analysis for clustering of genotypes.

Results and Discussion

Two factors analysis of variance (Table 1) revealed that there was a significant variation among the nine cultivars for growth as well as yield characters of broccoli and there were differences across the years for all the characters. But, the interaction between year and variety was not significant for majority of the traits except for days to 50% head initiation and yield per hectare pointing that the considerable environment interaction with genotype in the phenotypic expression was confined to only these two characters which are economically important.

Mean performance of cultivars in high elevated and tribal zone of Andhra Pradesh

Perusal of the mean table (Table 2) reflected that greater differences among the genotypes for days to 50% head initiation and yield per hectare. Maximum plant height was observed for Palam Vichitra (63.94 cm) on par with Palam Kanchan (63.29 cm) among the cultivars with an average mean height of 51.96 cm, while maximum

Table 1 : Analysis of variance for various traits in broccoli.

Source of variation	Df	PH	LL	LW	D50	CW	CD	YH
Replication	2	31.3*	21.47*	5.148**	18*	1895	4.33	746**
Year	2	123.1**	33.98**	16.91**	3341**	9225**	17.03**	3994**
Variety	8	512.5**	150.44**	6.277**	808**	25578**	53.95**	5360**
Year: Variety	16	1.5	0.02	0.012	333**	2059	0.58	90**
Residuals	52	6.2	5.17	0.91	5	1471	2.48	34

Table 2 : Mean performance of the broccoli cultivars for different traits.

S. no.	Genotype/Year	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Days to 50% head initiation	Head weight (g)	Head diameter (cm)	Yield/ha (Q)
1	Palam Kanchan	63.29^{a}	51.09^{a}	15.25^{abcd}	70.00^{d}	357.5^{bcd}	11.84^{b}	127.0^{e}
2	F1 Festival	46.43^{de}	41.96^{bcd}	16.48^{a}	63.94^{e}	291.5^{ef}	16.91^{a}	122.2^{e}
3	Palam Samradhi	42.19^{f}	36.10^{e}	14.83^{bcd}	83.22^{b}	335.5^{cde}	13.59^{b}	135.9^{d}
4	Oynasty	49.34^{cd}	41.27^{cd}	14.04^{cd}	68.67^{d}	365.6^{abc}	14.06^{b}	142.9^{cd}
5	Pusa Samradhi	45.29^{ef}	42.14^{bcd}	14.93^{bcd}	78.56^{c}	264.0^{f}	18.11^{a}	95.7^{g}
6	Saki	54.36^{b}	43.51^{bc}	15.55^{ab}	79.00^{c}	422.7^{a}	12.27^{b}	170.5^{a}
7	Palam Vichtira	63.94^{a}	45.10^{b}	15.49^{abc}	90.78^{a}	381.7^{abc}	12.29^{b}	148.7^{c}
8	NSC 105 B	51.28^{bc}	42.56^{bcd}	13.88^{d}	67.56^{d}	405.5^{ab}	17.49^{a}	160.7^{b}
9	Pusa KTS 1	51.51^{bc}	39.44^{de}	14.22^{bcd}	63.11^{e}	304.3^{def}	12.97^{b}	106.3^{f}
	Mean	51.96	42.57	14.96	73.87	347.58	14.39	134.43
1	2017-18	51.46^{b}	41.77^{b}	14.39^{b}	86.69^{a}	367.3^{a}	14.47^{ab}	148.1^{a}
2	2018-19	54.30^{a}	43.86^{a}	15.87^{a}	66.70^{c}	344.8^{ab}	15.14^{a}	130.3^{b}
3	2019-20	50.12^{b}	42.10^{b}	14.63^{b}	68.22^{b}	330.6^{b}	13.56^{b}	124.9^{c}
	Mean	51.96	42.57	14.96	73.87	347.58	14.39	134.43

Figures with similar letters are non-significant as per Tukey's Honest Significant Difference test.

leaf length was observed in Palam Kanchan (51.09 cm) followed by Palam Vichitra (45.10 cm). The highest leaf length was observed in F1 Festival (16.48 cm) on par with Saki (15.55 cm). Earliness is an important parameter that reduces the crop duration and fetches an early harvest. It was measured as days to 50% head initiation. Pusa KTS-1 took less number of days for 50% head initiation (63.11) on par with F1 Festival (63.94), while the cultivar Palam Vichitra took maximum number of days for 50% head initiation (90.78). There was a month difference between early and late types pointing out that there is a greater variation and scope for improving the earliness in broccoli. Head weight and head diameter are important yield components that determine the yield. Saki was having the highest head weight (422.7 g) on par with NSC 105 B (405.5 g), while the cultivar Pusa Samradhi had the lowest head weight (264.0 g). The cultivar Pusa Samradhi with low head weight had the maximum head diameter (18.11 cm), which was found to be at par with NSC 105 B (17.49 cm) and F1 Festival (16.91 cm). Yield is a very complex trait and selection of any genotype is greatly determined by its yielding capacity in a given environment. The cultivar Saki recorded the highest yield (170.5 g) followed by Pusa KTS 1 (160.7 g) while the cultivar Pusa KTS 1 had low yield (106.3 g).

The year wise mean performances (Table 2) showed that the performance of cultivars during 2017-18 and 2019-20 was at par with each other and different from 2018-19 for traits like plant height, leaf length and leaf width. The performance of cultivars had significant differences across all the three years for traits like days to 50% head initiation and yield per hectare which was justified by the significant year and genotype interaction revealed by ANOVA. The performance was almost similar for head weight and head diameter across the three years. None of these cultivars used in this experiment were not developed and selected for high altitude regions of Eastern Ghats in Andhra Pradesh, but, they performed well and produced good head weight, size and yield suggesting the suitability of the region for broccoli cultivation.

Estimation of genetic parameters

The presence of significant genetic variation among genotypes gives more scope for improvement in desirable

Table 3 : Estimation of genetic variability parameters for various traits in broccoli.

Character	GCV (%)	PCV (%)	Heritability (%)	GA % Mean
Plant height	14.41	14.70	96.17	29.12
Leaf length	9.44	9.93	90.34	18.48
Leaf width	5.15	6.34	66.14	8.64
Days taken to 50% head initiation	9.78	12.91	57.46	15.28
Head weight	14.24	16.20	77.31	25.79
Head Diameter	16.52	17.78	86.39	31.63
Yield/ha	17.94	18.27	96.46	36.30

characters through selection. Genetic variability study disclosed that the phenotypic coefficient of variance (PCV) was higher in level in comparison with the genotypic coefficient of variance (GCV) for all traits studied (Table 3), which pointed towards the significant effect of environment on the expression of the characters. These results agreed with the finding of Habib *et al*. (2013), Nandini *et al*. (2020), Kalia and Shakuntla (2002).

Moderate level of PCV and GCV (10-20%) was observed for plant height, days taken to 50% head initiation head weight, head diameter and estimated yield per hectare. The results revealed that these traits were predominantly under genetic control. Thus the selection for these traits could be effective in crop improvement programme of broccoli. Similar moderate PCV and GCV for plant height, days taken to 50% head initiation (Nandini *et al*., 2020), head diameter (Habib *et al*., 2013), yield per hectare (Habib *et al*., 2013). Low estimates of phenotypic coefficient variance and genotypic coefficient variance (<10%) were recorded in for leaf length and leaf width.

The variability presented in a population is the sum of heritable and non-heritable components. A higher value of heritability shows that the phenotype of specific character strongly reflects its genotype. In this investigation, the heritability in broad sense estimates were high for plant height, leaf length, head weight, head diameter and yield per hectare whereas moderate heritability was reported for days taken to 50% head initiation and leaf width (Table 3). High magnitude of genetic advance as percentage mean (>20%) was recorded in plant height, head weight, head diameter and yield per hectare and moderate level for days to 50% head initiation, leaf length whereas low magnitude was observed for leaf width. High heritability integrated with high genetic advance reveals that the heritability is due to additive gene effect and selection could be effective based on good phenotypic performance. Low heritability integrated with low genetic advance reveals that environment shows more impact on expression of the particular character and selection would be ineffective. Similar findings were reported by Nandini *et al*. (2020). Hence, improvement of head yield per hectare in broccoli would be successful through phenotypic selection for these characters. Broad sense heritability is an excellent measure to estimate the extent of genotypic variability. Burton (1952) stated that genotypic coefficient of variation mutually with heritability would give the maximum picture of the progress expected from selection, and it serves as a functional guide in understanding the proportion of total variation due to genotype.

Trait association

In general, extent of genotypic correlation coefficients was the higher than the subsequent phenotypic correlation coefficients for the characters positively associated with yield representing less environmental control on these traits. In this study, the yield per hectare showed a significant positive phenotypic correlation plus genotypic correlation with head weight. The plant height was evinced significant positive alliance with leaf length and head weight whereas as significant negative association with head diameter (Table 4). Similar results were reported by Nandini *et al.* (2019).

Principal Component analysis

Three years pooled mean values of the 9 broccoli cultivars were subjected to calculate the Principal Component Analysis (PCA) whereas, Principal Components (PCs) estimated from the correlation matrix. PCA is an expressive method which shows the pattern of covariation of traits among the individuals (Rhodes and Martins, 1972). PCA produces Eigen vectors for each principle component axis and eliminates extremely inter-correlated nature of prevalent variations, thereby decreases the dimensions of multivariate data (Koundinya *et al*., 2016).

Out of the seven PCs, the first three PCs with Eigen value >1.0 were selected as per Kaiser Criterion. Therefore the reduced descriptor dimensionality was three. In general, the first PC explains the maximum variation. Thus, here, the first PC with Eigen value 2.8417

Table 4 : Phenotypic and Genotypic correlation coefficients for various characters in broccoli.

Character		Plant height	Leaf length	Leaf width	Days taken to 50% head initiation	Head weight	Head diameter	Yield/ha
Plant height	P	1.000						
	G	1.000						
Leaf length	P	0.759**	1.000					
	G	0.865**	1.000					
Leaf width	P	0.130	0.332	1.000				
	G	0.179	0.254	1.000				
Days taken to 50% head initiation	P	0.227	-0.022	0.145	1.000			
	G	0.216	0.006	0.303	1.000			
Head weight	P	0.468*	0.196	-0.119	0.217	1.000		
	G	0.524**	0.346	-0.241	0.258	1.000		
Head Diameter	P	-0.661**	-0.374*	-0.024	-0.281	-0.380*	1.000	
	G	-0.734**	-0.359	-0.049	-0.298	-0.555**	1.000	
Yield/ha	P	0.302	0.100	-0.037	0.269	0.913**	-0.293	1.000
	G	0.315	0.137	-0.044	0.272	0.975**	-0.340	1.000

P = Phenotypic correlation coefficient; G = Genotypic correlation coefficient.

Table 5 : Eigen values, proportion of variance, cumulative variance and character loading of four Principal components (PCs).

PC	Eigen value	Proportion of variance (%)	Cumulative variance (%)	Characters associated
1	2.8417	40.6	40.6	Plant height (0.516), Leaf length (0.398), Head weight (0.489), Head diameter (-422), Yield per hectare (0.433)
2	1.725	24.6	65.2	Yield per hectare (-0.488)
3	1.1336	16.2	81.4	Leaf width (0.754), Days taken to 50% head initiation (0.631)

contributed 40.6% variation to the sum variability among the nine cultivars for diverse growth and yield traits. The second PC (1.725) was responsible for 24.6% variation and the third PC (1.1336) generated 16.2% variation. These three PCs collectively accorded 81.4% to the whole variability present in the broccoli cultivars for the observed the traits (Table 5).

Each PC associates with some of the characters and the variation explained by a particular PC is due to those characters. The first PC presented the maximum variation and was associated with the growth and yield components *viz*., plant height, leaf length, head weight and head diameter. Similarly, the second PC had correlation with yield per hectare which had 24.6% variation. The traits associated with the first two PCs had maximum variation suggesting that these characters contributed maximum to the variation and selection based on these characters would be useful. Leaf width and days taken to 50% head initiation were responsible for 16.2% variation due to their association with PC3.

Loading plot of characters and score plot genotypes were laid on principle component axes 1 and 2. Loading plot represents the relationship among the characters. The characters located in same direction are positively correlated while the variables in opposite direction are negatively correlated. Loading plot explained the positive correlation between plant height and leaf length; head weight and yield per hectare; and negative correlation between plant height and head diameter. These correlations were supported by the Pearson correlation coefficients presented in Table 4. The position of genotypes on score plot gives the understanding of their genetic relatedness to each other. The genotype Palam Kanchan with the maximum plant height and leaf length located far from all the other genotypes suggesting its diverse nature. Similarly the genotype Palam Samradhi also stood apart from other genotypes. The genotypes Oynasty, Palam Vichtira and NSC 105 B were closely

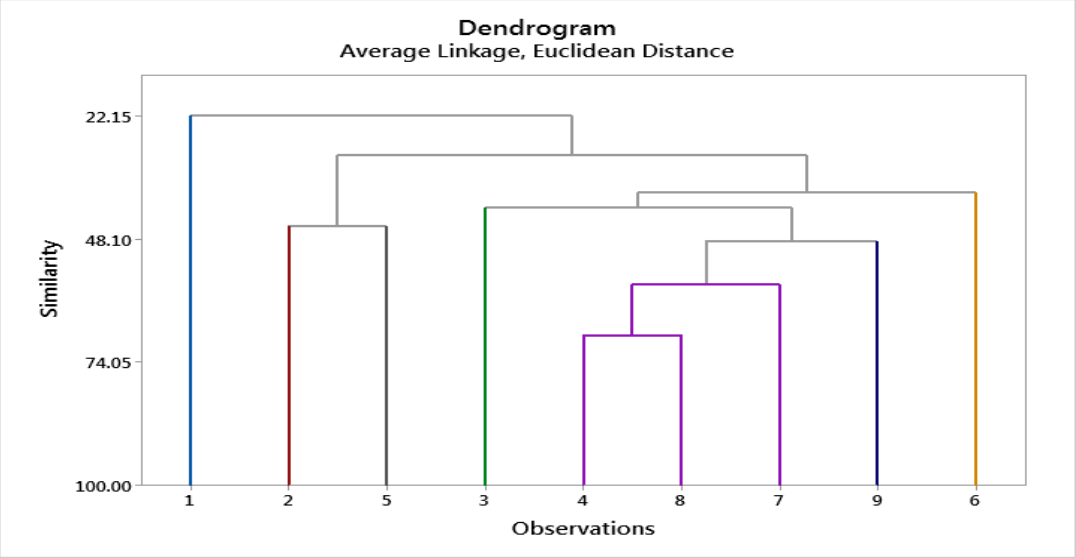

Fig. 1 : Dendrogram showing cluster classification of genotypes (1 to 9 are genotypes as per Table 2).

Table 6 : Cluster classification of broccoli cultivars.

Cluster Number	Genotypes
Cluster1	Palam Kanchan
Cluster2	F1 Festival
Cluster3	Palam Samradhi
Cluster4	Oynasty, Palam Vichtira, NSC 105 B
Cluster5	Pusa Samradhi
Cluster6	Saki
Cluster7	Pusa KTS 1

Table 7 : Inter cluster distances among the broccoli cultivars.

	Cluster1	Cluster2	Cluster3	Cluster4	Cluster5	Cluster6
Cluster2	4.8620					
Cluster3	5.6157	4.0895				
Cluster4	3.6584	3.4930	2.9275			
Cluster5	5.3133	3.0637	3.2353	3.5837		
Cluster6	3.5390	4.7567	3.7721	2.7115	5.3176	
Cluster7	4.1194	3.5671	3.7030	2.6525	3.5505	4.6254

located depicting their genetic closeness.

Moreover, loading plot and score plot are superimposed and complimentary to each other. The genotypes in each quarter of loading plot tend to have high mean values of the characters presented in the corresponding quarter on the loading plot (Koundinya *et al.*, 2016). The genotypes Palam Vichtira and Palam Kanchan in first quarter of score plot had high mean values for the characters in the first quarter of loading plot namely plant height and leaf length (Table 2). Similarly, the genotype F1 Festival in quarter-2 of score plot had the maximum leaf width, the trait in the quarter-2 of loading plot. The quarter-3 of score plot had the genotype Palam Samradhi which had maximum days for 50% flowering (quarter-3). The genotype Saki in quarter -4 of score plot had maximum head weight and yield per hectare corroborated to the fourth quarter of loading plot. Previously Koundinya *et al.* (2016) presented similar results in okra.

Cluster analysis

PCA only is not enough to study the genetic diversity amongst the existing germplasm unless it is accompanied with cluster analysis which categorizes the genotypes into divergent clusters. Such a study allows the selection of genetically divergent parents to attain highly heterotic hybrids (Moll *et al.*, 1962) and desirable recombinants in the segregating generations (Sharma and Prasad, 2010).

Cluster analysis classified the nine genotypes into 7 divergent clusters at 50% similarity (Fig. 1). Cluster classification of genotypes is presented in Table 6. Cluster-4 had three genotypes (Oynasty, Palam Vichtira and NSC 105 B) while all remaining clusters had single genotype. The genotype, Palam Kanchan was the highly diverse genotype followed by Saki and Palam Samradhi. This indicted the high diversity among the genotypes. These results were corroborated with score plot of genotypes obtained through PCA. Inter cluster distances

are presented in Table 7. The clusters-1 and 3 had high inter cluster distance (5.6157) followed by clusters- 5 and 6 (5.3176) while lowest inter cluster distance was found between clusters-4 and 7 (2.6525). It could be interpreted that clusters-1 and 3 were highly divergent clusters and hybridization between these two would give heterotic hybrids or transgressive segregates in segregating generations.

Conclusion

The sufficient amount of genetic variability was present for growth and yield traits. The mean performance of broccoli genotypes over three years in high altitude tribal zone of Andhra Pradesh during *Rabi* suggested that the prevailing weather conditions support the cultivation of broccoli in this area. High heritability (>60%) combined with high genetic advance (>20%) for plant height, days taken to 50% head initiation, head weight, head diameter along with yield per hectare indicated that these traits are commanded by additive gene action. So, straight selection possibly followed for the improvement of broccoli for these traits. The head weight had a positive direct effect on estimated yield per hectare. The traits associated with the first two PCs *viz*., plant height, leaf length, head weight, head diameter and yield/ha had maximum variation suggesting that these characters contributed maximum to the variation and selection based on these characters would be useful. The genotype, Palam Kanchan was the highly diverse genotype followed by Saki and Palam Samradhi. Based on inter cluster distance the genotypes Palam Kanchan (cluster-1) and Palam Samradhi (cluster-3) were highly diverse genotypes. Hybridization between these two would give superior variants in segregating generations.

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