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SHORT COMMUNICATION

Molecular Characterization of Cabbage (*Brassica oleracea* L. var. *capitata*) Genotypes using RAPD Markers

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The genetic relationship among sixteen genotypes of cabbage were studied using RAPD markers. Cabbage genotypes were collected from IARI Research Centre, Kattarine, Kullu and Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan. A total of 17 primers were used to generate RAPD profiles, out of these 7 primers gave reproducible banding patterns. A total of 22 bands were obtained and all were polymorphic with two cultivar specific bands. The percentage of primer polymorphism was 100%. Similarity index was computed based on Jaccard's similarity coefficient and used for cluster analysis based on UPGMA. At 62% similarity level sixteen genotypes were grouped into two clusters. RAPD technology could be useful for identification of different genotypes as-well-as accessing the genetic similarity among different accessions of cabbage.

Key Words: Cabbage, Genetic relationships, RAPD markers

A characteristic feature of living organisms is the immense natural variability present for various characteristics in most populations. A wide range of variability present in a species, always provides a better chance of selecting desirable genotypes. (Vavilov, 1951). Estimates of genetic diversity are important in designing crop improvement programme for management of germplasm. Assessment of genetic variation existing in a crop species is vital for revising strategies appropriate for directed genetic enhancement of the crop. Morphological characters such as leaf shape, leaf colour, flower colour and fruit colour which are robust qualitative genetic traits have been used in the past to study genetic variation and characterization of crop genotypes. Due to interaction of quantitative traits with environment, these markers were not found suitable for assessing genetic diversity.

Molecular markers thus show significant advantage in the analysis of genetic diversity. These are not influenced by the environment and proved to be useful in providing estimates of amount of genetic diversity, relationship with other variables and understanding evolution process. PCR based markers have been proved useful for genetic diversity studies. The RAPD markers are simple, fast and arbitrary markers and are appropriate for formulation of strategies for effective management of germplasm collections and estimation of diversity (Virk *et al.,*1995; Caetano *et al*.,1999; Chong *et al*., 2005; Gong *et al*., 2007; Cheng *et al*., 2009; Hoque *et al*., 2013; Sharifova *et al*., 2013). Among vegetable crops, cabbage being largely cultivated in hilly areas of H.P. Present study therefore carried out to estimate the genetic diversity between different cabbage genotypes using RAPD markers.

The plant material was obtained from IARI Research Centre Kattarine, Kullu and Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, sown in glass house at Biotechnology Department in 2010. One gram of green leaves were collected and used for the DNA isolation (Table 1).

Total genomic DNA from each variety was isolated by CTAB method with slight modification using a rapid method (Doyle and Doyle 1999). The extracted DNA was purified with 10 µg/ml RNase A for 1 hour at 37^{o}C to remove the RNA. The purified DNA was dissolved in TE buffer and stored at -20^{o}C freezer for further use. Seventeen RAPD primers (Sigma Aldrich) were used to characterize 16 cultivars of cabbage. The PCR reaction having 25.0 µl volume mixture contained 17 µl sterile deionized water, 2.5 µl Taq DNA polymerase buffer (2X) with 1.5 mM $MgCl_2$(GENEI), 1.25 µl of each 2.5 mM dNTP (GENEI), 0.25 µl Taq DNA polymerase (1unit) (GENEI), 2 µl primer (13.32 µM/reaction) and 2.0 µl sample DNA (approx. 40-50 ng). The reaction mixture was subjected to the following thermal profile for amplification in a thermocycler (BioRad Amplification

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Table 1. List of cabbage genotypes used in DNA study (RAPD)

S. No.	Name of cabbage genotypes	Abbreviation	
1	Golden Acre	GA	IARI, Kattraine, Kullu
2	Best of all	BA	IARI, Kattraine, Kullu
3	BC-79	BC	IARI, Kattraine, Kullu
4	Green Emperor	GE	IARI, Kattraine, Kullu
5	Green Europium	Ge	IARI, Kattraine, Kullu
6	KGAT-3	KG	IARI, Kattraine, Kullu
7	Pusa Drum Head	P	IARI, Kattraine, Kullu
8	Darl Cabbage	DC	IARI, Kattraine, Kullu
9	EC-30191	EC	Dr YSPUHF, Nauni
10	AC-204	AC	Dr YSPUHF, Nauni
11	NO-29	NO	Dr YSPUHF, Nauni
12	NO-4	4	Dr YSPUHF, Nauni
13	Giddeon	GI	Dr YSPUHF, Nauni
14	Green Kid	GD	Dr YSPUHF, Nauni
15	Cabbage Mangla	CM	Dr YSPUHF, Nauni
16	General Cabbage	GC	Dr YSPUHF, Nauni

System): 4 min at 94^{o}C for initial denaturation, followed by 40 cycle of 30 second DNA denaturation at 94^{o}C, 1.0 min annealing at 36 ºC and 2 min at 72^{o}C for extension. A final extension step was done at 72^{o}C for 7 min. Electrophoresis was done to visualize the PCR amplified product on 1.4% agarose gel. The amplified fragments were visualized by staining with ethidium bromide.

Data analysis was carried out only for those primers which give scorable banding patterns for the genotypes under study. Co-migrating bands were considered to represent the same locus and thus treated as a same band while scoring. Presence of an amplified product was designated as "1" and absence was marked as "0". Intensity of the amplified products was not taken into account while scoring. NTSYS-pc, version 2.02 (Numerical Taxonomy System Exterer Software) was used to perform cluster analysis of the complete RAPD data (Rohlf, 2000). Similarity between accessions were estimated using the Jaccard coefficient, calculated as J = A/ (N-D) where, A is the no. of positive matches (i.e. the presence of bands in both the samples), D is the no. of negative matches (*i.e*. the absence of bands in both the samples) and N is the total sample size including the no. of matches and unmatches. Similarity estimates were analysed by unweighted pair group method with arithmetic averages (UPGMA) and the resulting clusters were expressed as dendrogram in the cabbage genotypes.

A total of 22 bands were obtained with seven RAPD primers and all were found to be polymorphic. Two unique bands were obtained in genotype No-4 and Darl Cabbage with primers OPB-03 and OPB-07 respectively. Percentage of total polymorphic bands was 100% (Table 2). Using Jaccard's similarity coefficient maximum similarity was found to be 0.8235 between the variety Cabbage Mangla and Best of all, whereas the least similarity was found to be 0.1429 between the variety No-4 and Green Emperor. Fig. 1 shows dendrogram which was divided into two main clusters 1 and 2. The cluster 1 was further divided into two subclusters (1a and 1b). The subcluster 1a Golden acre and BC-79 genotypes were grouped together. The subcluster 1b further divided into sub-subclusters in which sub-subcluster 1ba including Best of all, Cabbage Mangla, General cabbage, Giddeon and Green kid genotypes. The subcluster Ibb includes Darl cabbage, Green Emperor, No-4, EC-30191, Green Europium and AC-204 genotypes. The main cluster B including the genotypes KGAT-3 and Pusa Drum Head.

In an attempt to examine the potential of RAPD markers for the ability to asses the variability in cabbage genotypes, 7 primers were used in genotyping. Cansian and Echevarrigaray (2000) have also used RAPD markers to characterize 16 commercial cultivars of cabbage. They selected 18 random decamer primers from the set of 100 primers, produced a total of 195 bands, out of which 105 (54%) were polymorphic that ranged from 100 to 2500 bp. Dias *et al*. (1991) used 13 random decamer primers for assessing genetic variation within *Brassica campestris* cultivars using RAPD markers. They obtained 15 unique bands with a number of primers namely OPE-01, OPF-07, OPK-01, OPK-02, OPK-03, OPK-04, OPN-01 to distinguish YSPb, Tobin, Debra and Pont toria cultivars. Zang and Zang (2014) evaluated genetic diversity in 21 cultivars of Chinese kale (*Brassica oleracea* L. var. *alboglabra* Bailey) by using rapid amplified polymorphic DNA and sequence-related amplified polymorphism markers. A total of 104 bands were detected by 11 RAPD primers, of which 66 were polymorphic in nature. Similarly, Panwar *et al*. (2009) have also studied the potential use of RAPDs in characterizing 25 genotypes of cauliflower with 12 reproducible primers.

Present study revealed that informative primers can be efficiently used for diversity analysis among cabbage genotypes at molecular level. A high value of similarity

Table 2. Total number of amplified fragments and number of polymorphic fragments generated by PCR using seven random decamer oligonucleotide primers

S.No.	Primer Name	Sequence (5′-3′)	Total Number of Amplified bands	Total Number of Polymorphic amplified bands	Total Number of Monomorphic amplified bands	Genotype specific bands	Polymorphism ratio, percentage (%)
1.	OPB-01	GTTTCGCTCC	2	2	0	0	100
2.	OPB-02	TGATCCCTGG	4	4	0	0	100
3.	OPB-03	CATCCCCCTG	2	1	0	1	50
4.	OPB-04	GGACTGGAGT	4	4	0	0	100
5.	OPB-05	TGCGCCCTTC	3	3	0	0	100
6.	OPB-07	GGTGACGCAG	4	3	0	1	50
7.	OPB-09	TGGGGGACTC	3	3	0	0	100
	Total		22	20	0	2	

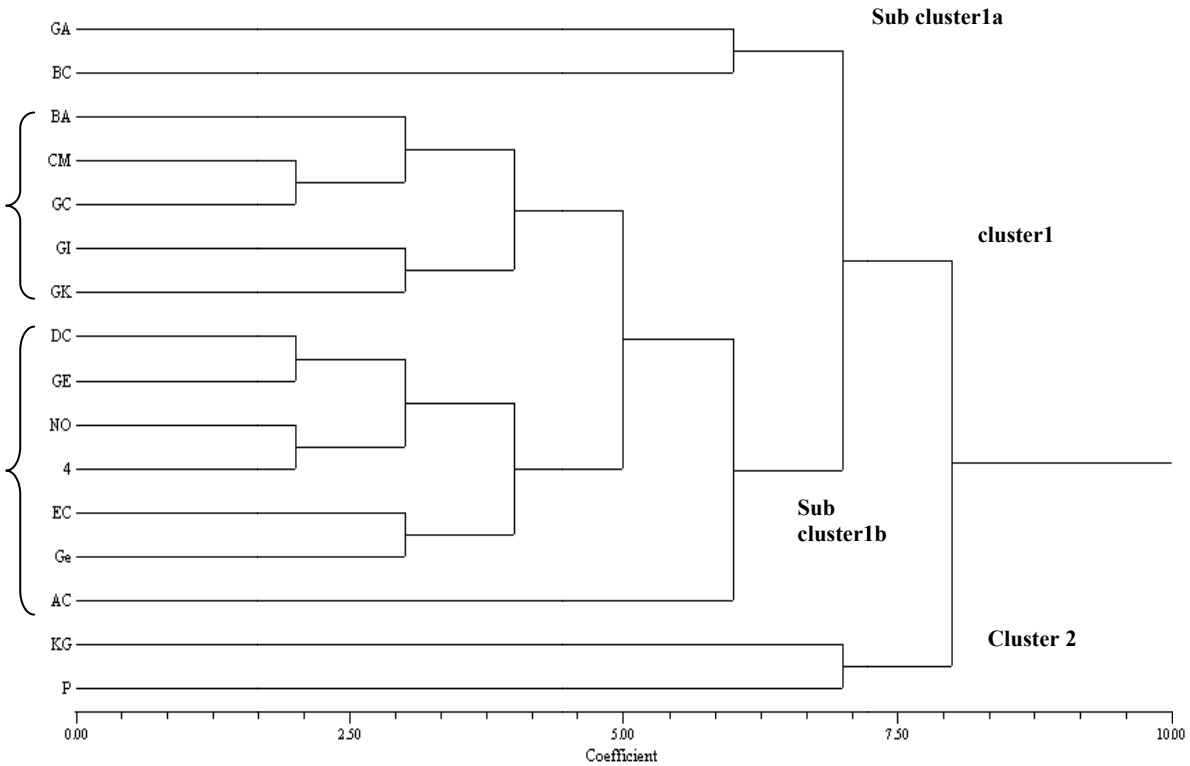

Fig. 1. Dendrogram obtained from RAPD analysis among cabbage (*Brassica oleracea* L.var. *capitata*) genotypes

index (0.8235) between the genotypes Cabbage Mangla and Best of all was obtained using Jaccard coefficient. The genotypes thus have same parent of origin. A least value of similarity index (0.1429) was found between the variety No-4 and Green Emperor revealed that genotypes different parents of origin. From the dendrogram it was clear that the genotypes Golden acre, BC-79, Best of all, Cabbage Mangla, General cabbage, Giddeon and Green kid, Darl cabbage, Green Emperor, No-4, EC-30191, Green Europium and AC-204 have emerged from same parents because they grouped in cluster A. The genotypes KGAT-3 and Pusa Drum Head have emerged from other parent, thus grouped into cluster B. Based on the aforesaid findings, it is concluded that cabbage genotypes collected from Katraine and Nauni are genetically diverse and the breeders can choose the most diverse parents for breeding programs for its germplasm collection and management.

References

Caetano AG, BJ Bassam and PM Gresshoff (1999) DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. *Biotechnology* **9:** 553-557.

Cansian RL and YS Echeverrigaray (2000) Discrimination among cultivars of cabbage using RAPD markers**.** *Hort.Sci.* **35:** 1155-1158.

Cheng Y, J Geng, J Zang, Q Wang and X Hou (2009) Construction of a genetic linkage map of non-heading Chinese cabbage. *J. Genet. Genomics* **36:** 501-508.

Chong L,Y Tian, C Bo and R Yunying (2005) Optimization of RAPD reaction system for cabbage. *J. Acta Agri. Shanghai* **3:** 114-117.

Dias JS, MB Lima, KM Song, AA Monteiro, PH Williams and TC Osborn (1991) Molecular taxonomy of Portuguese trochunda cabbage and kale landraces using nuclear RFLPs. *Euphytica* **58:** 221-229.

Doyle JJ and JL Doyle (1999) Isolation of plant DNA from fresh tissue. *Focus* **12:**13-15.

Gong X, X Wang and H Shen (2007) Seed genetic purity testing of F_1 hybrid with molecular markers. *Seed Sci. Technol.* **35:** 477-486.

Hoque ME, N Huq and NJ Moon (2013) Molecular diversity analysis in potato (*Solanum tuberosum*) through RAPD markers. *SAARC J. Agric.* **11:** 95-102.

Panwar A, G Aggarwal and DK Srivastava (2009) Moleculor characterization of cauliflower (*Brassica oleracea* L. var *botrytis*) genotypes using RAPD. In: Proc. National Symposium on Plant Propagation, Conservation, Modification and Characterization. Abstract, pp. 58-59.

Rohlf JF (2000) NTSYSpc Numerical Taxonomy and Multivariate Analysis System, Version 2.1. User Guide. Departement of Ecology and Evolution, State University of New York. Stony Brook, NY.

Sharifova S, S Mehdiyeva, K Theodorikas and K Roubos (2013) Assessment of genetic diversity in cultivated tomato (*Solanum lycopersicum* L.) genotypes using RAPD primers. *J. Horti. Res.* **21:** 83-89.

Vavilov NI (1951) The origin, variation, immunity and breeding of cultivated plants. *Botanica* **12:** 1-364.

Virk PS, BV Ford-Lloyd, MT Jackson and H John Newbury (1995) Use of RAPD for the study of diversity with plant germplasm collections. *Heredity* **74:** 170-179.

Zhang J and LG Zhang (2014) Evaluation of genetic diversity in Chinese kale (*Brassica oleracea* L. var. *alboglabra* Bailey) by using rapid amplified polymorphic DNA and sequence-related amplified polymorphism markers. *Genet. Mol. Res.* **13 :** 3567-3576.