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Microsatellite maker based intervarietal variability study in Indian cauliflower

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ABSTRACT

The present study was conducted during 2015-2016 to assess molecular diversity in cauliflower. A total of 48 genotypes comprising early, mid-early and mid-late were characterized using 26 SSR markers. The polymorphic primers produced bands ranging from 2.00 to 5.00. A total of 76 alleles with an average of 2.24 alleles per locus were amplified in 48 cauliflower genotypes. Heterozygosity for individual loci among 48 genotypes ranged from 0.00 to 0.98. The UPGMA dendrogram allocated 48 cauliflower genotypes into six major clusters having maximum 8 genotypes (DB 15, CC 522, Selection 1-2, Maghi, PusaHimjyoti, AL 15, CC 35 and DC 431) in cluster I. The molecular markers employed in this study were effective in discriminating the 48 cauliflower genotypes.

Key words: *Brassica oleracea* var. *botrytis,* variety, diversity, SSR.

INTRODUCTION

Cauliflower (*Brassica oleracea* var. *botrytis* L.; 2n=2x=18) is the most important winter vegetable of Brassicaceae family. India, being the second largest producer in the world with an estimate production of 8.98 million tonnes from 0.41 million hectare area accounting to 5.3% share in total vegetables production. The productivity (19.6 MT/ha) is very low in comparison to other countries (Egypt, 29.00 MT/ha; Germany, 24.03 MT/ha) (Anonymous, 1). For successful genetic improvement of a character, the nature and magnitude of variability present in the gene pool for different characters and their relationship is responsible (Kumar *et al*., 7). *Brassica oleracea* represents primary gene pool by itself and therefore, the nature and magnitude of variability present in it will determine the success of its genetic improvement. Identification of a genotype is important for accurate classification of cultivar. Genetic diversity is considered essential and pre-requisite for hybridization programme in obtaining progenies with desirable characters like higher yield, earliness, good quality and disease resistance. Such study preferred the genetically divergent parents in obtaining desirable combinations among segregating generations. Traditional characterization of cauliflower genotypes/breeding lines on the basis of morphological description are laborious (Cansian and Echeverrigaray, 3). The DNA markers have become increasingly recognized tools over other for cultivar identification, genotype validation and estimation of genetic variability where the DNA is not affected by environmental factors or the developmental stage of plants (Choudhary *et al*., 4). Currently, a range of dominant and co-dominant molecular markers are available for assessing genetic diversity in plants. RAPD marker has been employed for fingerprinting of various varieties in *Brassica oleracea* and gene diversity evaluation (Zhang and Zhang, 15). Louarn *et al.* (9) characterized 59 cultivars of *B. oleracea* by using SSR markers. Most of the accessions have polymorphism information content (PIC) of more than 0.5. Zhao *et al.* (14) analyzed the genetic diversity of 57 *Brassica oleracea* genotypes with 14 simple sequence repeat primer pairs. The overall genetic similarity was 0.74 and 0.83 for normal cauliflower and loose curd accessions, respectively. Therefore, assessing the nature and degree of genetic variability/ similarity of genotypes at molecular level will be helpful in management of genetic resources. Keeping in view of the above facts, the present study was undertaken to assess the molecular diversity among varieties of cauliflower using simple sequence repeats (SSR) markers.

MATERIALS AND METHODS

The current research was undertaken during autumn-winter season of 2015-16 at Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi under normal sowing condition. The experimental materials for the present study consisted of 48 diverse accessions of cauliflower (Early, Mid-early and Mid-late) collected from various parts of India and maintained in the Division. The details of these genotypes are given in Table 1. All these 48 genotypes were transplanted in the field during autumn-winter season of 2015-16. The experiment was laid out in

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Randomized Complete Block Design (RCBD) with three replications. Each genotype was planted in a bed size of 4.5 × 3.0 m with a spacing of 50 × 50 cm (Row × Plant) and standard package of practices were followed to raise a good crop. Fresh leaves of all genotypes were collected at 45-60 days after sowing from five random plants of each 48 genotypes. DNA was isolated from young expanding leaves of 48 genotypes using the modified CTAB method (Doyle and Doyle, 5). A total of 26 C-genome derived SSR primers known to express polymorphism among 48 cauliflower genotypes were used. The amplified fragments were resolved on 3% agarose gel. The amplified fragments were scored manually for their presence (denoted as '1') and absence (denoted as '0') for each primer. The similarity matrices were subjected to unweight pair group method of arithmetic average (UPGMA) for cluster analysis by using Darwin Software 6 version (Posada and Crandall, 12). For the analysis of SSR marker in Power Maker software version 3.25, band pattern for each of the microsatellite markers were recorded for each genotypes by assigning a letter to each band for e.g. alleles were numbered as A1, A2 etc. Utilizing this software, number of alleles and values of gene diversity and PIC (Botstein, 2) of each locus from 34 SSR markers were calculated. PIC was calculated by using the formula PIC = 1− Σ $(Pi)^2$, where Pi is the proportion of the population carrying the i^{th} allele.

RESULTS AND DISCUSSION

The SSR markers were used to assess the genetic diversity among 48 cauliflower genotypes. A total of 34 SSR primers were screened and 26 primers were amplified with scorable polymorphic alleles which were used for further studies. The number of bands amplified by each of 26 primers ranged from 2.00 to 5.00. The amplification profile for marker BoGMS 1145 and BoGMS 1432 were presented in Fig.1. A total of 76 alleles with an average of 2.24 alleles per locus were amplified in 48 cauliflower genotypes. Heterozygosity for individual loci among 48 genotypes ranged from 0.00 to 0.98. The major allele frequency ranged from 0.13 to 1. Maximum numbers of alleles (5) were observed for the primers BoGMS 0162 and BoGMS 1432. The primers viz., BoGMS 0037, BoGMS 0282, BoGMS 0631, BoGMS 0661, BoGMS 0710, BoGMS 1259, BoGMS 1264, BoGMS 1412, BoGMS 1467, BoGMS 1565 amplified 3 alleles. PIC value for 26 polymorphic markers in the present study varied from 0.08 (BoGMS 1570 and BoGMS 0408) to 0.71 (BoGMS 0282 and BoGMS 1162), with an average PIC value of 0.41.The UPGMA dendrogram obtained by 26 SSRs distributed 48 genotypes into six major clusters as shown in Fig. 2. The cluster I from bottom predominantly consisted of 8 genotypes (DB 15, CC 522, Selection 1-2, Maghi, Pusa Himjyoti, AL 15, CC 35 and DC 431). The cluster II comprised of

Table 1. Cauliflower genotypes employed for diversity analysis.

S. No.	Early genotypes	S. No.	Mid-early genotypes	S. No.	Mid-late genotypes
1	Ageta Early Chawkra	1	Pusa Sharad	1	AL 15
2	CC 12	2	DC 306	2	CC 22
3	CC 13	3	DC 308	3	CC 32
4	CC 14	4	DC 310	4	CC 35
5	CC 15	5	DC 310-22	5	DB 15
6	DC 33-8	6	DC 312	6	DC 402
7	DC 351aa	7	DC 321	7	DC 431
8	DC 41-5	8	DC 325	8	DPCa 2
9	Early Kunwari	9	DC 326	9	HR 6-5-1
10	Pusa Deepali	10	DC 333	10	Improved Japanese
12	Pusa Kartiki	12	DC 371	12	Pant Gobhi 2
11	Pusa Meghna	11	DC 340	11	Maghi
13	Selection 7	13	DC 383	13	Pusa Himjyoti
14	Selection 18	14	DC 385	14	Pusa Shukti
15	Selection 67	15	DC 522	15	Pusa Paushja
16	Selection 71			16	Selection 1-2
				17	Selection 18-19

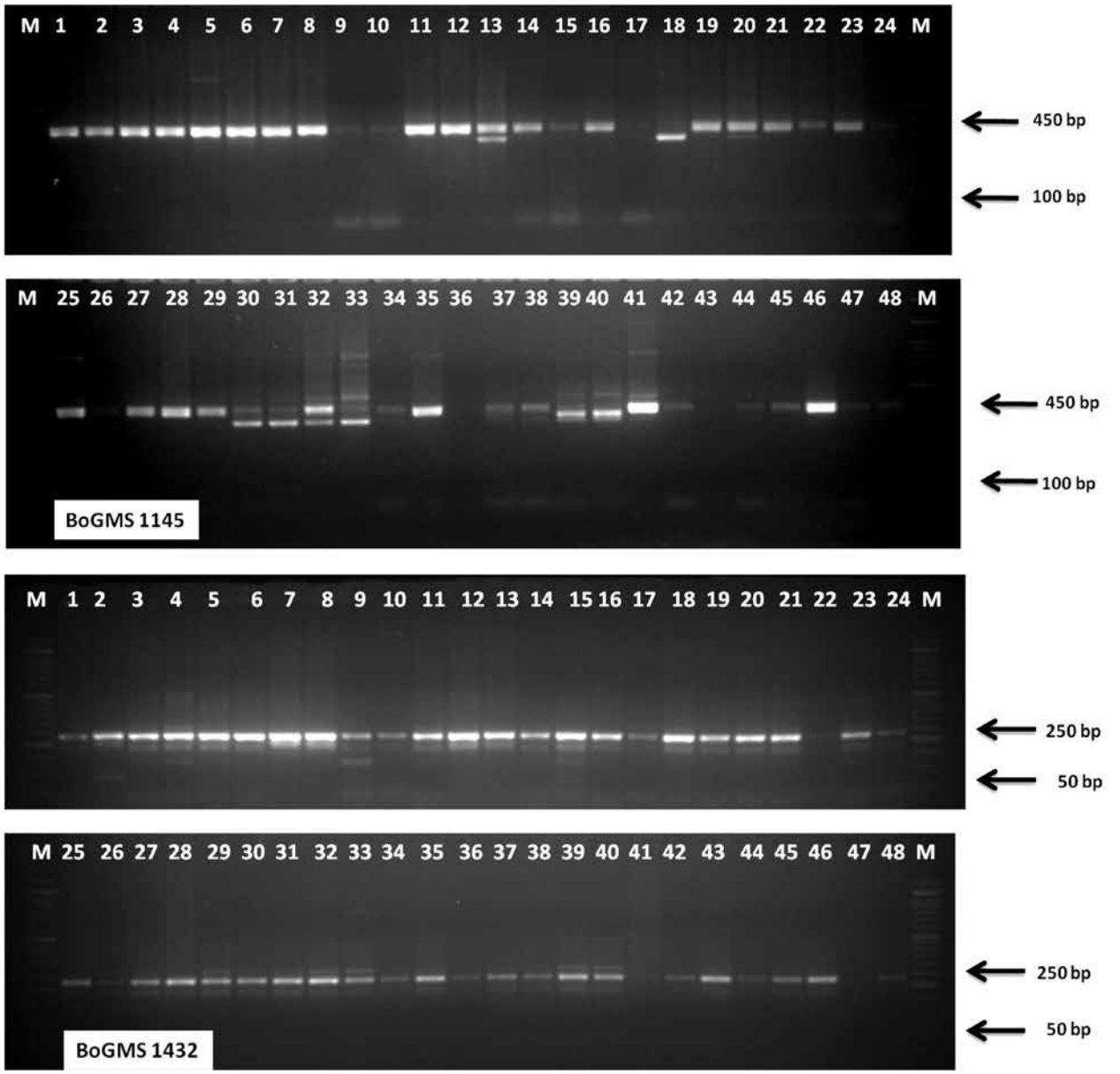

Fig. 1. SSR allelic profiling for genetic diversity of 48 cauliflower genotypes, where M, 100 bp Ladder; Lane 1-48, Selection 67, Pusa Meghna, Selection 71, Pusa Kartiki, DC 33-8, Pusa Deepali, CC 12, Selection 7, DC 351aa, Selection 18, CC 15, CC 13, Ageta Early Chawkra, DC 41 -5, CC 14, Early Kunwari, DC 306, DC 308, Pusa Sharad, DC 310, DC 310-22, DC 312, DC 321, DC 325, DC 326, DC 340, DC 371, DC 383, DC 385, DC 522, DC 333, Pusa Himjyoti, Maghi, Pant Gobhi 2, HR 6-5-1, Pusa Paushja, Selection 1-2, Improved Japanese, CC 35, DC 431, AL 15, Selection 18-19, DB 15, Pusa Shukti, DC 402, DPCa 2, CC 32, CC 22.

fourteen genotypes, namely Pusa Meghna, DC 351 aa, DC 340, CC 32, CC 22, DC 312, DC 325, Pant Gobhi 2, Pusa Paushja, Improved Japanese, DC 306, Selection 18, Selection 18-19 and Pusa Shukti while the cluster III had only two genotypes (DPCa-2 and DC 402). Cluster IV included seven genotypes *viz.,* DC 310, DC 310-22, Early Kunwari, Pusa Sharad, DC 321, DC 41-5, and CC 14. Four genotypes namely, DC 333, CC 13, Ageta Early Chawkra and DC 308 comprised cluster V. Cluster VI consisted of 13 genotypes, namely DC 371, DC 385, CC 12, DC 383, HR 6-5-1, Selection 71, Pusa Kartiki, Pusa Deepali, Selection 67, DC 326, DC 33-8, Selection 7 and CC 15.

The SSR marker has been found to be useful and robust tool for detecting genetic diversity. SSR

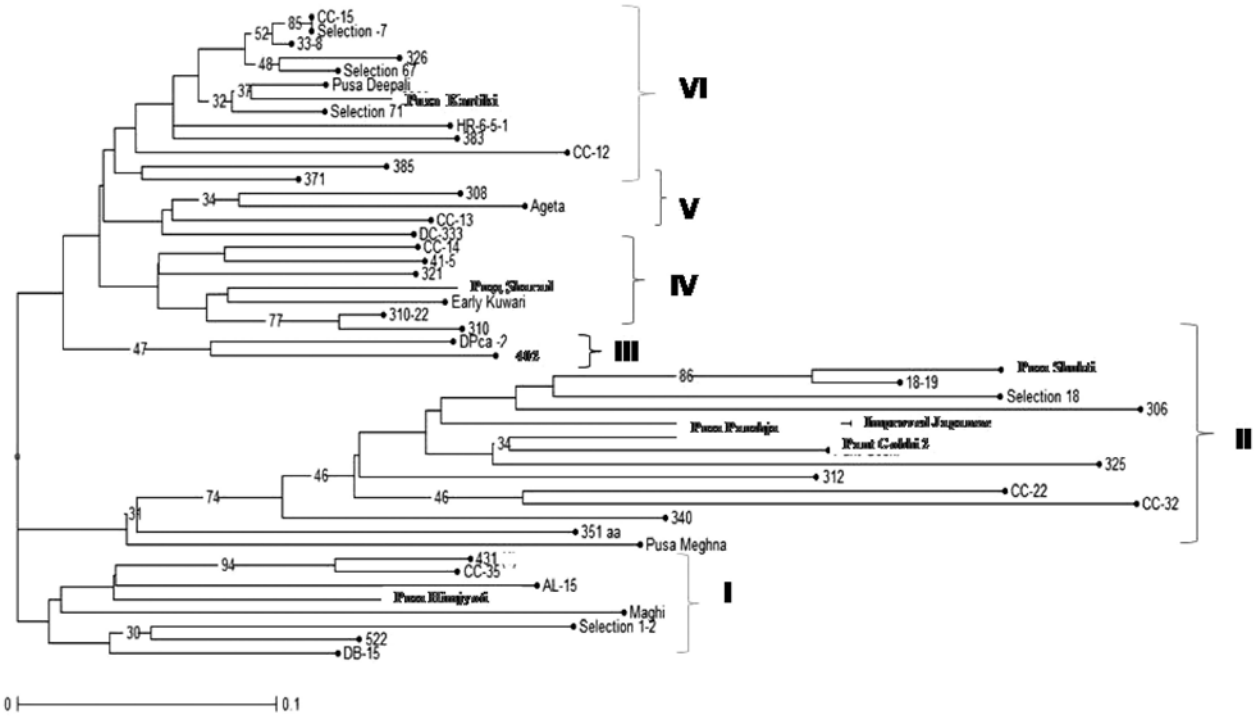

Fig. 2. Dendrogram showing Jaccard's dissimilarity using UPGMA cluster analysis demonstrating association among 48 cauliflower genotypes.

markers are highly polymorphic, multi-allelic, co-dominant, polymerase chain reaction (PCR) based, easily reproducible and widely distributed among the genome (Powell *et al.,* 13). The high level of polymorphism (88.57%) generated by SSRs in the present study is in evident with previous studies (Lowe *et al*., 10) and may be due to slipped strand mis-pairing (Levinson and Gutman, 8), which occurs more often than deletion and insertion events (Powell *et al*., 13). The average PIC is an ideal index to measure polymorphism. A PIC value greater than 0.5 indicate loci of high polymorphism, values between 0.25 and 0.5 indicate loci of intermediate polymorphism and less than 0.25 indicate loci of low polymorphism (Ge *et al.*, 6). In the present study, the maximum PIC values were observed in BoGMS 1162 and BoGMS 0282. The minimum PIC was recorded in BoGMS 0870, BoGMS 0738, BoGMS 0408, BoGMS 1486, BoGMS 1570 and BoGMS 0355. The average PIC value of 0.41 was recorded for 34 SSR markers which indicate that the loci display intermediate polymorphism and occurrence of a narrow gene pool among the genotypes. This may due narrow genetic base in the genotypes under study. The high polymorphic markers detected can be implicated for monitoring of genes introgression to desirable genetic backgrounds and identifying desirable genotypes for the development of hybrids.

The effectiveness of SSR markers in assessing the genetic diversity are in agreement with Plieske and Struss (11). In the present study, molecular markers including genomic-SSR were used to assess the diversity of cauliflower. Considerable genetic diversity among the genotypes at molecular levels was observed. This is important for germplasms classification, management, and further utilization as reported by Louarn *et al.* (9). The application of this technique may alleviate some of the confusion of cultivar identity associated with morphological characteristics and multiple cultivar registrations. In summary, the classification of cauliflower genotypes based on the SSR marker profile may be useful in discriminating the closely related cauliflower genotypes. The genotypes can be selected based on their relatedness during crossing programme of cauliflower.

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