Comparison of traditional grow-out test and DNA-based PCR assay to estimate F_1 hybrid purity in cauliflower

Arpita Pattanaik[1,2], D. C. Lakshmana Reddy[2], S. Ramesh[3] and Aswath Chennareddy[4,*]

[1]Division of Biotechnology, Centre for Post-Graduate Studies, Jain University, Bengaluru 560 011, India
[2]Division of Biotechnology, Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Hesaraghatta, Bengaluru 560 089, India
[3]Department of Genetics and Plant Breeding, College of Agriculture, University of Agricultural Sciences, GKVK, Bengaluru 560 065, India
[4]Division of Floriculture and Medicinal Crops, Indian Institute of Horticultural Research, Bengaluru 560 089, India

Cauliflower (*Brassica oleracea*) is a cool-season crop belonging to the Brassicaceae family. Use of morphological differences between true-to-types and off-types in grow-out test (GOT) is the basic method for hybrid purity analysis. Traditional GOT is costly, tedious, time consuming and environment sensitive. To increase the speed and accuracy of genetic purity testing of hybrids, recent advances in DNA markers have shown promise. In the present study, the purity of cauliflower hybrid (NBH Tania-815) was assessed by traditional GOT and advanced molecular marker systems. The experiment was carried out by mixing 95% F_1 hybrids with 5% female parents, individually in the sample sets of 400, 300, 200, 100, 80 and 40. For each sample size, PCR-based assay and GOT were carried out to check the hybrid purity. In the PCR-based assay, 220 pairs of SSR markers were screened, with 32 markers showing parental polymorphism including one co-dominant marker (BrgMS565). The purity level was determined by the co-dominant marker. A minimum sample size of 100 was standardized to confirm the hybrid purity as it showed the same result with that of higher sample sizes (200, 300 and 400). Hence, it is proposed that molecular marker-based hybrid purity assessment may serve as an effective substitute to traditional GOT.

Keywords: Cauliflower, grow-out test, hybrid purity, PCR assay.

CAULIFLOWER (*Brassica oleracea* var. *botrytis*; family Brassicaceae, $2n = 18$) is one of the widely used vegetables in the world due to its wide adaptation, high-yielding nature, long shelf-time, and good economic returns. It is a cool-season crop, usually cultivated between October and April in India. The total cultivated area of this crop is 1.382 million hectares worldwide, with an annual production of over 24.175 million tonnes[1]. The maintenance of genetically uniform inbred lines and commercial hybrids that are true to their type is an essential prerequisite in the production and marketing of hybrids. Identifying breeding lines and determining F_1 hybrid purity are consequential quality controls in vegetable breeding and seed production. In cauliflower, F_1 hybrid selection aims for earliness, high yield, better curd quality with regard to compactness and colour, uniform maturity, and resistance to insects, diseases and unpropitious weather conditions[2]. Self-incompatibility is commonly utilized for hybrid cauliflower seed production, but the formation of self-inbred seed often contaminates hybrid seed production. The presence of female selfs can also reduce the yield and economic value through loss of hybrid purity. For characterizing a good quality seed, hybrid purity is the most important parameter and it is performed to confirm any deviation from the genuineness of the variety. For seed certification and commercialization of hybrid, maintenance of high level of genetic purity assessment is mandatory.

The traditional approach for assessing genetic purity by grow-out test (GOT) is based on the identification of morphological characteristics at various stages of plant growth. This approach is subject to influence by environmental factors and is time-consuming. Biochemical methods like isozyme analysis and seed protein electrophoresis cannot discriminate closely related genotypes because of limited polymorphism and environmental influence. Furthermore, they do not give precise estimates of genetic distances among cultivars[3]. Therefore, there is growing need for a novel method in hybrid purity testing which can provide accurate, rapid and cost-effective results. Employing DNA-based markers for hybrid purity testing can overcome the drawbacks of morphological or biochemical markers as marker analysis is based on the genotype of the hybrids eliminating environmental variations and permitting identification of hybrid purity even at the seed level. The simple sequence repeat (SSR) markers are co-dominant, highly informative, reproducible and the most reliable for assessment of hybrid purity. The heterozygosity of the hybrids can be effortlessly recognized by the presence of both the parental

*For correspondence. (e-mail: aswathiihr@gmail.com)

alleles[4]. In recent years, SSR markers have been utilized for genetic purity testing in various field and vegetable crops such as rice[5,6], cotton[7,8], sunflower[9], cabbage[10,11], cauliflower[12] and tomato[13].

Even though molecular marker plays an important role in identifying hybrid purity, there are no guidelines available on the use of a specific number of markers, and sample size for 90%, 95% and 99% purity in comparison with GOT, where 400 seeds were used as minimum sample size for 95% purity. There is an urgent need for this method to be standardized for large scale 'hybrid purity test'. Hence the present study was undertaken with the objective of optimizing the minimum sample size that can be used for genetic purity assessment. The results of genetic purity determined through SSR markers were then compared with those of GOT performed on different sample sizes.

Materials and methods

Plant material

The materials used for the study comprised of cauliflower F_1 hybrid NBH Tania-815 and its parental lines, which were developed in Noble Seeds Pvt Ltd, India. The hybrid plant showed erect, creamy white curd colour and curd partially covered by inner leaves. The female parental line showed semi erect, yellowish curd colour and curd covered by inner leaves; while the male parental line showed erect, creamy white curd colour and curd partially covered by inner leaves (Table 1).

GOT analysis

To validate the conformity of molecular marker-based estimates of selfed or off-type plants with the actual phenotypic data, the experiment was carried out by pooling 95% F_1 hybrids mixed with 5% female parents, individually in the following sample sets of 400, 300, 200, 100, 80 and 40. All the six sample sizes along with the parental lines of NBH Tania-815 were sown in the tray with 20×20 matrix GOT layout for 30 days. One-month-old seedlings were transplanted to the plots. Measures like proper irrigation, fertilization and insect management were carried out during the plant growth. Purity evaluations were examined based on major morphological traits mentioned the in DUS (distinctness uniformity and stability) guidelines[14], including plant habit, leaf shape, leaf colour, curd compactness and curd colour throughout the growing season (Table 1). For each sample size, both the PCR-based assay and GOT were carried out to check the hybrid purity. The selfed/off-type plants identified through SSR marker analysis were further validated based on the morphological characters. The experiment was repeated thrice and the mean per cent hybrid purity based on both PCR-based assay and GOT was calculated as follows

$$\text{Hygrid purity (\%)} = \frac{\text{Total number of plants} - \text{number of off-types}}{\text{Total number of plants}} \times 100.$$

DNA isolation and quantification

Genomic DNA was extracted from leaves of parental lines and hybrids from each sample size (400, 300, 200, 100, 80 and 40) using the modified cetyltrimethylammonium bromide method[15]. The quality and quantity of isolated genomic DNA was checked using 0.8% agarose gel electrophoresis and Gene Quant UV Spectrophotometer (GE Health Care Bio-Sciences Ltd, Bengaluru) respectively. Finally, DNA was diluted with double-distilled water to a concentration of 20 ng/µl for PCR analysis.

SSR marker polymorphism analysis

A total of 220 pairs of SSR markers were used to examine the polymorphism in bulk of DNA extracted from ten seedlings of each female and male parent of cauliflower NBH Tania-815 hybrid[16–20]. Polymorphic SSRs were chosen for the hybrid purity testing of NBH Tania-815. Sequences of all SSR markers were obtained from public sources: http://www.brassica.info/ssr/SSRinfo.htm. DNA amplification was carried out in a total volume of 20 µl reaction mixture containing 10X Taq polymerase buffer, 5 pmol of each primer, 1 mM dNTP, 3U of Taq polymerase (Bangalore Genie Pvt Ltd, Bengaluru, India), and 20 ng of template genomic DNA. Amplification was performed in a thermocycler (model TC-5000; Bibby Scientific (Asia) Limited, Hong Kong) with the following conditions: denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 1 min and a final extension at 72°C for 10 min. Amplified DNA fragments were separated in a 3% (w/v) agarose gel containing 0.5 µg/ml ethidium bromide in 45 mM Tris-borate (1 mM) EDTA buffer, pH 8.0. Next, 20 µl of PCR products was loaded into the well after adding 5 µl of loading dye (50% (w/v) glycerol and 50% (w/v) bromophenol blue). A 100 bp DNA ladder (3B bioscience, Spain) was used as the molecular standard. Electrophoresis was carried out at 70V for 3 h and gels were visualized under UV transilluminator (Syngene, USA) and documented using UV-Pro gel documentation system.

Results

Genetic purity assessment using GOT and SSR markers

In GOT, purity evaluation was carried out based on morphological traits. In the present study, plants from six

Table 1. Morphological characters used to identify the selfed/off-types during grow-out test

Morphological characters	Female parent of NBH Tania-815	Male parent of NBH Tania-815	NBH Tania-815
Plant habit	Semi erect	Semi erect	Erect
Leaf: attitude	Semi erect	Semi erect	Erect
Leaf: length and shape	Long and narrow	Medium and broad	Medium and broad
Leaf: colour and waxiness	Dark green and medium	Dark green and light	Dark green and light
Curd: covering by inner leaves	Not covered	Not covered	Partially covered
Curd: shape and size	Narrow and medium	Dome and medium	Dome and medium
Curd: colour	Yellowish	Creamy white	Creamy white
Curd: compactness	Medium	Medium	Compact
Curd: knobbing	Medium	Medium	Fine
Curd: maturity group	Early	Mid early	Mid early

different sample sizes (400, 300, 200, 100, 80 and 40) of NBH Tania-815 were studied individually to determine if they were true-to-type for ten morphological characters in GOT. Of the ten morphological characters analysed, nine distinguished the impurities in six different sample sizes (Table 1). Among these, plant habit, leaf shape, curd compactness and curd colour exhibited maximum variation (Figure 1). The characters of few individuals which showed variation from the standard characters were identified as selfed/off-type. The mean percentage of hybrid purity in GOT assay for 400, 300, 200, 100, 80 and 40 sample sizes of NBH Tania-815 was calculated to be 99.4, 99.3, 99.3, 99.6, 99.5 and 100 respectively (Table 2).

In the PCR-based assay, a total of 220 pairs of SSR markers were used to detect polymorphic loci between the parental lines. Out of 220 marker pairs, 32 showed clear polymorphism among the parental lines (Table 3). These 32 polymorphic markers were then tested on the hybrid population to detect the heterozygosity of hybrids. A single marker (BrgMS565) was found to be co-dominant and resulted in amplification of 190 bp female-specific amplicon (FSA) as well as 220 bp male-specific amplicon (MSA) that were present in the hybrid NBH Tania-815 (Figure 2). Further, this co-dominant marker was tested on all the six sample sizes and mean percentage was calculated per sample size. In the 400 hybrid sample size mean percentage of hybrid purity in SSR analysis was calculated to be 99.3 (Table 2). Similarly, in case of 300, 200, 100, 80 and 40 hybrid sample sizes, the mean percentage of hybrid purity was calculated to be 99.2, 99.3, 99.6, 99.5 and 100 respectively (Table 2). Visually, it is difficult to assess the hybrid genetic purity due to environmental conditions. While SSR analysis is based on genomic level, which cannot be influenced by the environment. As in the present study, two plants, i.e. plant no. 14 from 400 sample size and plant no. 67 from 300 sample size were phenotypically similar to F_1 hybrid, but at the genotypic level, they were off-types/selfed (Figure 3).

Duration involved in determining the purity of cauliflower hybrid

GOT, based on the assessment of morphological characters of plants took around 70 days to detect the selfed/off-types in the six sample sizes. The PCR-based assay, however, took around 48 h to detect the level of admixtures, suggesting a time saving approach for testing hybrid purity against the admixture of selfed plants as well as off-types (Table 4).

Discussion

In seed production, genetic purity of hybrids and varieties is of critical importance. Contamination is possible due to several reasons, such as pollen shedders, outcrossing and physical mixtures during the consistent multiplication of the harvested material. In this study, 3000 individuals were evaluated using SSR markers as well as GOT in order to compare both test methods and thus to confirm the purity of NBH Tania-815 for future marketing.

In the present study, although a total of 220 pairs of markers were screened, 32 SSR markers showed parental polymorphism. Such a result, which indicates a high similarity of genetic background between the parents, is due to the relatively narrow genetic base in *B. oleracea*[21]. The development of DNA markers is highly desirable to facilitate the purity testing of such cultivars derived from closely related parental lines.

For making the hybrid commercially successful, it should be pure. The genetically pure hybrid will only perform better in a consistent manner, or it would show poor performance. Hence to check the genetic purity of the produced hybrid, it is mandatory to use molecular markers. Traditionally, GOT is being used for genetic purity testing, which requires a complete season. Also, it is labour-intensive as well as sensitive to environmental changes and therefore is not totally a true-to-type method to assess genetic purity[22]. Due to the above reason, there is unavailability of hybrid seeds for immediate cultivation

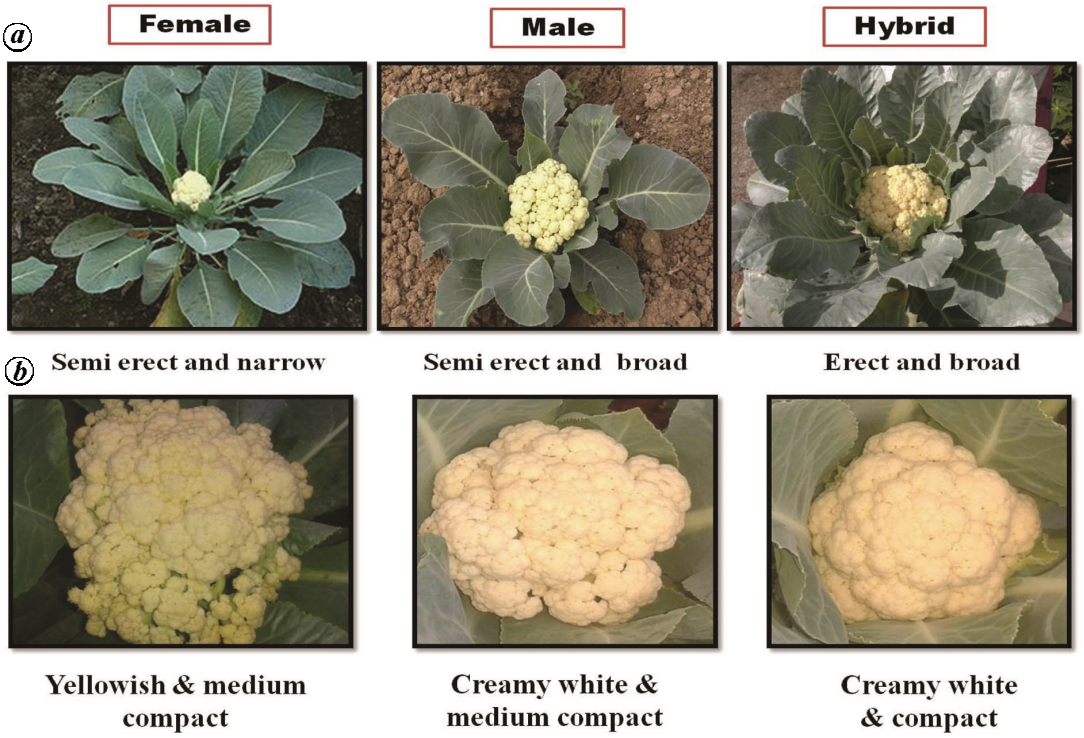

Figure 1. Variability in morphological parameters of cauliflower hybrid NBH Tania-815 and its parents. ***a***, Plant habit and leaf shape. ***b***, Curd colour and compactness.

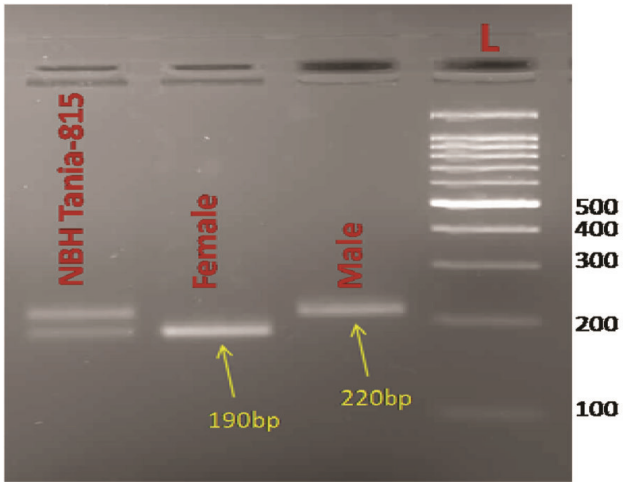

Figure 2. Amplification profile of cauliflower hybrid NBH Tania-815 and its parents with co-dominant SSR maker BrgMS565 resolved on 3% agarose gel. L, 100 bp DNA ladder.

which leads to the extra cost of storage, and hence an overall increase in the hybrid seed cost.

Among various DNA-based markers available for genetic purity testing, SSRs are preferred as they are co-dominant and convenient. It has been demonstrated that a single co-dominant marker is sufficient to discern false hybrids in purity assessment[23,24]. Nevertheless, discrepancies between the results obtained using different SSR markers are not infrequent. In this study, only a single SSR marker (BrgMS565) was found to be co-dominant, which was used for hybrid purity assessment to reconfirm the reliability of the marker. The plants which were recognized as off-types based on GOT analysis were also recognized as off-types through SSR analysis. The results of the field GOT and SSR marker tests were comparable. The molecular marker-based assay detected a higher percentage of impurities compared to traditional GOT assay[25]. Molecular markers identified some additional impurities, which could not be done during morphological markers. This illustrates the better discriminatory power and efficiency of SSR markers in genetic purity assessments. These markers could even precisely detect residual heterozygosity in the plant. Similar results have been reported by other researchers[6,8,24,26]. The present study helps to critically assess the hybrid purity of the variety using molecular marker-based advanced technology to get true-to-type seeds to the farmers.

Previously for GOT, 400 seedlings were used as the minimum sample size for testing genetic purity. However, maintenance of such huge sample size was tedious and labour-intensive. Alternatively, we have standardized by testing six different sample sizes, viz. 400, 300, 200, 100, 80 and 40. So instead of 400 sample size, 100 seedlings are sufficient to confirm the hybrid purity of cauliflower, as they showed results comparable with higher sample size. Marker analysis showed consistent results with GOT. However, GOT takes around 70 days to estimate genetic purity and this technique is both laborious

Table 2. Comparison of hybrid purity assessment on the basis of morphological and molecular markers with different sample sizes in cauliflower

Sample size	Replicate 1				Replicate 2				Replicate 3			
	GOT Assay	Purity (%)	SSR analysis	Purity (%)	GOT assay	Purity (%)	SSR analysis	Purity (%)	GOT assay	Purity (%)	SSR analysis	Purity (%)
Sample size 400 mixed with 5% admixture (+ ++)												
Expected	380	99	380	99	380	99.47	380	99.47	380	99.75	380	99.47
Observed no. of true-to-type hybrids	376		376		378		378		379		378	
Observed no. of off-types/selfed	4		4		2		2		1		2	
Mean of GOT assay						377.6 (99.4%)						
Mean of SSR analysis						377.3 (99.3%)						
Sample size 300 mixed with 5% admixture (+ ++)												
Expected	285	99.3	285	99.3	285	98.6	285	98.6	285	100	285	99.7
Observed no. of true-to-type hybrids	283		283		281		281		285		284	
Observed no. of off-types/selfed	2		2		4		4		0		1	
Mean of GOT assay						283 (99.3%)						
Mean of SSR analysis						282.6 (99.2%)						
Sample size 200 mixed with 5% admixture (+ ++)												
Expected	190	99.5	190	99.5	190	100	190	100	190	98.43	190	98.43
Observed no. of true-to-type hybrids	189		189		190		190		187		187	
Observed no. of off-types/selfed	1		1		0		0		3		3	
Mean of GOT assay						188.6 (99.3%)						
Mean of SSR analysis						188.6 (99.3%)						
Sample size 100 mixed with 5% admixture (+ ++)												
Expected	95	100	95	100	95	99	95	99	95	100	95	100
Observed no. of true-to-type hybrids	95		95		94		94		95		95	
Observed no. of off-types/selfed	0		0		1		1		0		0	
Mean of GOT assay						94.6 (99.6%)						
Mean of SSR analysis						94.6 (99.6%)						
Sample size 80 mixed with 5% admixture (+ ++)												
Expected	76	100	76	100	76	99.7	76	99.7	76	100	76	100
Observed no. of true-to-type hybrids	76		76		75		75		76		76	
Observed no. of off-types/selfed	0		0		1		1		0		0	
Mean of GOT assay						75.6 (99.5%)						
Mean of SSR analysis						75.6 (99.5%)						
Sample size 40 mixed with 5% admixture (+ ++)												
Expected	38	100	38	100	38	100	38	100	38	100	38	100
Observed no. of true-to-type hybrids	38		38		38		38		38		38	
Observed no. of off-types/selfed	0		0		0		0		0		0	
Mean of GOT assay						38 (100%)						
Mean of SSR analysis						38 (100%)						

Table 3. Polymorphic microsatellite (SSR) makers and their sequences used in the study

Marker	Sequence ID	Motif	Sequence (5′–3′)
BrgMS1238	AC189204	(AGTTT)4	F: TGAAGACAAATGCGGAGAAGT R: TCACCTCGATTGAACATTCTTG
BrgMS430	AC189187	(AT)11	F: CCCACCATATACCGTCACTTTT R: GTTGTATTCTATTGCCGTTAGGGT
BrgMS783	AC189217	(AT)11	F: CCAGGCTAAGTGATGATTCC R: AAACTAAGGAAGAAAGAAGCCC
BrgMS782	AC189219	(AT)11	F: AATGGTTCTCTGATGGCTTTGT R: ACCTCTCCTGGTCCTGTATTTG
BrgMS629	AC189440	(AT)14	F: GTGCTTTCTGCGTTATTTCTCA R: TTACGACCACCAACTAGCAAAA
BrgMS598	AC189475	(AT)16	F: ACCCGGAATACCTCAAAAGATT R: GTTACATGCAGCCAACACATTT
BrgMS570	AC189514	(AT)16	F: TAGTTGCTAGTGTGCCCATTTA R: ACTCTCAGTCGTGCCAAATAAG
BrgMS565	AC189521	(ATA)19	F: AACCCTCTTGAAAACATAGGCA R: CTCCTTGTCGTGATCCTTTACC
BrgMS354	AC189267	(CT)20	F: TTGATGTAAGATGACCAGTGCC R: TCAACAAGACTGCCGTATCCTA
BrgMS635	AC189428	(CTC)7	F: GTGTTTCTCTTCAACGCCTTTT R: CACAAAGAATCCCCACAGATTT
BrgMS1237	AC189204	(TA)10	F: ATCAAAAGATGCAGGGAGAGAG R: GTCCTCAATGGATTACACATGC
BrgMS309	AC189332	(AG)15	F: ACGTTACACCCTCCTAAGACCA R: GTTTTACGGTTTGCTCGAAAAG
BrgMS216	AC189431	(ATG)9	F: ACACGGGCTACAAAACAAGAGT R: ATGCTTCTGATTTAACCTGGGA
BrgMS3	AC190049	(GAA)11	F: GTCGTCCACTTCCTCATCTTCT R: TGGTGACTTCCCCTTTAATTTG
BrgMS932	AC189431	(GAT)8	F: CACATGCAACAAAACTAGAGTCG R: GTAACCTGGGAAGTGAAGATGG
BrGMS4509	AC189431	(TA)8	F: AATTCAGTCCCTACATCCAAGG R: ATATGGAGAGGCCAATGAGTGA
BrgMS579	AC189501	(TAGA)5	F: GCTTTCTTCAGATCCTCCTTGA R: CCTTTCACTTGATCGTTCTTCC
BrgMS455	AC172869	(TC)16	F: AACTCGTGCGCTAAGTAACCTC R: CATGTGTGTGGGGAGAGATAGA
BrgMS287	AC189355	(TG)19	F: TGGGTCTCAGTTTCCATTTTCT R: TGCTTGTGAATCTTTGTGTGTG
BrgMS651	AC189404	(CTCA)5	F: ACGCGAGAGTGTGAGAGAGAG R: TAGGCCCATTGAAACCATCTAC
BrgMS477	AC155339	(GA)16	F: TTTCGGATCATAGCTGTTCCA R: TCAGAGAAACAAACACCAAGC
BrgMS1474	AC189230	(TA)10	F: ATTTGTTTGTGGCCTTCTGTTC R: GATTTATTTGTTGAGTTGCGGG
BrgMS767	AC189237	(TA)10	F: GGTAAATGGTTAGTTGACCCGA R: GGAAGGCAAGCACCAAATAG
BrgMS773	AC189230	(TA)13	F: ATGTGGATGGAGAATCCGTG R: CCTGCATGTGACTAAGAGAAGGT
BrgMS1466	AC189218	(TA)18	F: GAACACCTTACTTTGACGCACA R: CCCTGTTTGTCTCTCCTATGCT
BrgMS628	AC189441	(TA)23	F: TCTGAGGTTTTCCAAAGGCTAC R: TACACATACAAGCGCAACAACA
BrgMS240	AC189404	(TA)24	F: TTAATCGGGTCTTTACGGTCTG R: CTGGCAAAATCTGTGACCACTA
BrGMS3274	AC232569	(TC)8	F: ACGCACTTAGCGAGGTAATGAT R: GGGTCACTGATAAAGATCTCCTC
BrgMS1501	AC189303	(TCCCG)4	F: ATTGGGCAGCTATTTTCAAGAC R: TTCTCTCACACTGGTTTCATCG
BrGMS4502	AC189208	(TCT)5	F: CATTGTATCTTTGGCTCCTCCT R: CTTGCTCTAAGAAGAGCGTGGT
BrgMS1600	AC189532	(TCT)7	F: CGTCGATTTAGGGGAGATACAG R: GTTTGAGCTTGACCTTGTTGTG
BrgMS719	AC189317	(TCT)9	F: TTGTTGTTTCTTGACATGGAGG R: GTTACCGAACCGTAGTGGTTTC

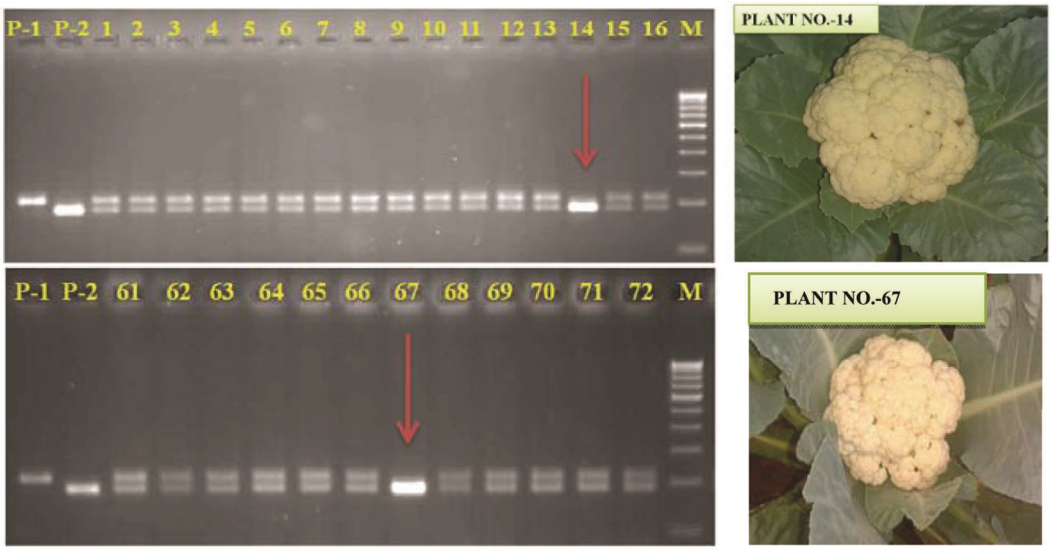

Figure 3. Phenotypically similar F_1 hybrid showing genotypically off-types/selfed.

Table 4. Time taken to determine the purity of cauliflower hybrid NBH Tania-815 by GOT and molecular-based assay

	GOT	Molecular based assay
Duration	Around 70 days*	48 h (after seedling stage)†

*Several of the distinguishing characters appear only during the flowering and post-flowering stage, which delays the assessment of genetic purity. †SSR analysis done in the laboratory takes 48 h for genetic purity estimation, thus reducing the time involved in testing the genetic purity of hybrid seed lots.

and time-consuming, as several of the distinguishing characters appear only during the flowering and post-flowering stage. As a result, it often delays the whole process of decision-making, packaging and marketing of the commercial seeds. Thus, farmers do not get hybrid seeds at the right time for sowing, thus preventing the immediate cultivation of such seeds produced. The hybrid seed cost escalates due to additional investment in production and storage[24]. These limitations of GOT can be overcome efficiently by employing molecular markers. The SSR analysis can be done in the laboratory which takes 48 hours for genetic purity evaluation; thus it reduces labour, space and ultimately the time involved in purity testing.

Therefore, we can conclude that it is possible to differentiate cauliflower hybrids more accurately and efficiently from their parental lines and off-types/selfed using locus-specific allelic information through SSR markers. The genetic purity of the variety/hybrid assessed through SSR markers and field GOT indicates that there is similarity in the results, suggesting that molecular markers can be used as a supportive test for GOT. Further, implementation of marker analysis would be beneficial to the seed industry due to its cost-effectiveness. In addition, commercially viable genetic SSR markers could be used for routine evaluation of genetic purity of cultivars. The information on SSR markers generated in this study will be useful to the seed industry to choose appropriate marker combinations for assessing the genetic purity of crops.

1. FAO, FAOSTAT database collections. Food and Agriculture Organization of the United Nations, Rome, Italy, 2014; http://faostat.fao.org/default.aspx.
2. Crisp, P. and Tapsell, C. R., Cauliflower (*Brassica oleracea* L. var. *botrytis*). In *Genetic Improvement of Vegetable Crops* (eds Kallo, G. and Bergh, B. O.), Pergamon Press, Oxford, UK, 1993, pp. 157–178.
3. Ainsworth, C. C. and Sharp, P. J., The potential role of DNA probes in plant variety identification. *Plant Var. Seeds*, 1989, **2**, 27–34.
4. Naresh, V., Yamini, Rajendra Kumar, K. N. and Dinesh Kumar, V., EST SSR marker-based assay for the genetic purity assessment of safflower hybrids. *Euphytica*, 2009, **170**, 347–353.
5. Moorthy, K. K., Babu, P., Sreedhar, M., Sama, V. S. A. K., Kumar, P. N., Balachandran, S. M. and Sundaram, R. M., Identification of informative EST-SSR markers capable of distinguishing popular Indian rice varieties and their utilization in seed genetic purity assessments. *Seed Sci. Technol.*, 2011, **39**, 282–292.
6. Sundaram, R. M. *et al.*, Identification of informative SSR markers capable of distinguishing hybrid rice parental lines and their utilization in seed purity assessment. *Euphytica*, 2008, **163**, 215–224.
7. Dongre, A. B., Rout, M. P., Bhandarkar, M. R. and Meshram, K. J., Identification and genetic purity testing of F_1 hybrid using molecular markers. *Indian J. Biotechnol.*, 2011, **10**, 301–306.
8. Selvakumar, P., Ravikesavan, R., Gopikrishnan, A., Thiyagu, K., Preetha, S. and Manikanda Boopathi, N., Genetic purity analysis of cotton (*Gossypium* spp.) hybrids using SSR markers. *Seed Sci. Technol.*, 2010, **38**, 358–366.
9. Pallavi, H. M., Gowda, R., Vishwanath, K., Shadakshari, Y. G. and Bhanuprakash, K., Identification of SSR markers for hybridity and seed genetic purity testing in sunflower (*Helianthus annuus* L.). *Seed Sci. Technol.*, 2011, **3**, 59–66.

10. Liu, G., Liu, L., Gong, Y., Wang, Y., Yu, F., Shen, H. and Gui, W., Seed genetic purity testing of F1 hybrid cabbage (*Brassica oleracea* var. capitata) with molecular marker analysis. *Seed Sci. Technol.*, 2007, **35**, 477–486.
11. Liu, L., Liu, G., Gong, Y., Dai, W., Wang, Y., Yu, F. and Ren, Y., Evaluation of genetic purity of F_1 hybrid seeds in cabbage with RAPD, ISSR, SRAP and SSR markers. *Hortic. Sci.*, 2007, **42**, 724–727.
12. Verma, V. K., Kalia, P. and Prasanna, B. M., Genetic characterization of self-incompatible lines and strategies for heterosis breeding in cauliflower. *Int. J. Veg. Sci.*, 2017, **23**, 411–429.
13. Liu, L. W., Yan, W., Gong, Y. Q., Zhao, T. M., Liu, G., Li, X. Y. and Yu, F. M., Assessment of genetic purity of tomato (*Lycopersicon esculentum* L.) hybrid using molecular markers. *Sci. Hortic.*, 2007, **115**, 7–12.
14. UPOV, Guidelines for the conduct of tests for distinctness, uniformity and stability on cauliflower (*Brassica oleracea* L. var. *Botrytis*), 2009, UPOV TG/45/7.
15. Doyle, J. J. and Doyle, J. L., A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull*., 1987, **19**, 11–15.
16. Xu, J. *et al.*, Construction of an integrated genetic linkage map for the A genome of *Brassica napus* using SSR markers derived from sequenced BACs in *B. rapa*. *BMC Genomics*, 2010, **11**, 594.
17. Burgess, B. *et al.*, Identification and characterization of simple sequence repeat (SSR) markers derived *in silico* from *Brassica oleracea* genome shotgun sequence. *Mol. Ecol. Notes*, 2006, **6**, 1191–1194.
18. Louarn, S., Torp, A. M., Holme, I. B., Andersen, S. B. and Jensen, B. D., Database derived microsatellite markers (SSRs) for cultivar differentiation in *Brassica oleracea*. *Genet. Resour. Crop Evol.*, 2007, **54**, 1717–1725.
19. Tonguc, M. and Griffiths, P. D., Genetic relationships of *Brassica* vegetables determined using database derived sequence repeats. *Euphytica*, 2004, **137**, 193–201.
20. Zhao, Z., Gu, H., Sheng, X., Yu, H., Wang, J. and Cao, J., Genetic purity testing of loose-curd cauliflower hybrids using SSR markers and grow out test. *Seed Sci. Technol.*, 2012, **40**, 209–214.
21. Hu, J. and Quiros, C. F., Identification of broccoli and cauliflower cultivars with RAPD markers. *Plant Cell Rep.*, 1991, **10**, 505–511.
22. Hashizume, T., Sato, T. and Hirai, M., Determination of genetic purity of hybrid seed in watermelon (*Citrullus lanatus*) and tomato (*Lycopersicon esculentum*) using randomly amplified polymorphic DNA (RAPD). *Jpn. J. Breed.*, 1993, **42**, 367–375.
23. Yashitola, J., Thirumurugan, T., Sundaram, R. M., Naseerullah, M. K., Ramesha, M. S., Sarma, N. P. and Stone, R. V., Assessment of purity of rice hybrids using microsatellite and STS markers. *Crop Sci*., 2002, **42**, 1369–1373.
24. Nandakumar, N., Singh, A. K., Sharma, R. K., Mohapatra, T., Prabhu, K. V. and Zaman, F. U., Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. *Euphytica*, 2004, **136**, 257–264.
25. Keshavulu, K., Identification of microsatellite markers for distinguishing elite fine grain rice varieties and hybrids and their utilization in seed purity assessments, Ph D thesis, Tamil Nadu Agricultural University, Coimbatore, 2006, pp. 34–70.
26. Yun, X. Y., Zhang, Z., Yi-Ping, X. and Long-Ping, Y., Identification and purity test of super hybrid rice with SSR molecular markers. *Rice Sci*., 2005, **12**, 7–12.

ACKNOWLEDGEMENT. This research was supported by the Indian Institute of Horticultural Research, Bengaluru.

Received 11 October 2017; revised accepted 29 May 2018

doi: 10.18520/cs/v115/i11/2095-2102