Vegetable Science (2020) 47(2): 183-188

Confirmation of hybridity in DOGR hybrids of onion (*Allium cepa* L.) using SSR markers

Amar Jeet Gupta*, S. Anandhan, V. Mahajan, Snehal K. Kad and Major Singh

Received: January 2021/ Accepted: February 2021

Abstract

Molecular identification and hybrid purity testing in nine onion hybrids along with their seven parental lines was conducted at ICAR-Directorate of Onion and Garlic Research, Rajgurunagar, Pune (Maharashtra). Four out of ten microsatellite (SSRs- Simple Sequence Repeats) markers were found to be polymorphic. All four polymorphic markers revealed polymorphism between the male and female parents of one or more hybrids and was utilized for their hybridity testing. ACM016, ACM033 and ACM078 have been identified as the three diagnostic markers for testing the genetic purity of all the nine onion hybrids included in this study *viz*.; DOGR Hy-1, DOGR Hy-2, DOGR Hy-3, DOGR Hy-4, DOGR Hy-5, DOGR Hy-6, DOGR Hy-7, DOGR Hy-8 and DOGR Hy-50. These 10 SSR loci amplified a total 28 alleles in 9 hybrids and 7 parents with 2-4 alleles per loci. The average number of alleles per loci was found to be 2.8. The highest polymorphism information content (PIC value) was observed to be 0.7137 for the marker ACM093 and the three potential markers ACM016, ACM033 and ACM078 with PIC value 0.5874, 0.5949 and 0.6628, respectively were the best markers that could be used to differentiate the parental lines and utilized for hybridity testing. This study showed that SSR are reliable and effective molecular marker capable of detecting genetic purity of onion hybrids.

Keywords: Onion, hybridity, SSR markers, male sterile lines, F_1 hybrids

Introduction

Onion (*Allium cepa* L.) having chromosome number 2n=2x=16, belongs to *Alliaceae* family is an important edible *Allium* species and ranks second in value on the list of cultivated vegetable crops in the world. Onion is one of the oldest cultivated vegetable, recorded for over 4000 years. The yield and production in India during 2018 were 18.1 t/ha and 23.2 million tonnes (FAOSTAT 2018), respectively. India ranks second in the production of onion and about 70 varieties/ hybrids have been released through public sector organization. Maharashtra is the leading state in onion production followed by Uttar Pradesh and Odisha. At present, onion breeding programme mainly focuses on improvement of existing cultivars. Area under onion cultivation in India is mainly covered by open pollinated varieties (OPVs) whereas in developed countries, areas under hybrids have increased significantly (Gupta and Singh 2016). It is difficult to develop onion hybrids as it requires 4-6 years for isolating maintainer lines and then developing hybrids. Cytoplasmic male sterility is known in many crops and is commonly used to produce hybrid seeds. The most widely used source of male sterility for hybrid seed production in onion was conditioned by the sterility inducing cytoplasm (S) and a single nuclear restorer gene in its recessive condition (ms/ms) (Jones and Emsweller 1936, Jones and Clarke 1943). S cytoplasm has been widely used to produce hybrid onion seed for most of the world's major onion producing areas. CMS is widely used because of the relatively simple inheritance of nuclear male fertility restoration and stable expression of male sterility across a range of temperature.

Hybrid seeds are produced only by the male sterile plants, so the proportion used to provide pollen should be minimal (Williams and Free 1974). Hybridity test is done to confirm any deviation from authentication of the variety during its multiplication. For seed certification of all the foundation and certified hybrid seeds, genetic purity test is most essential. Higher genetic purity is an important parameter for the commercialization of any hybrid seeds. The genetic purity during multiplication stages is prone to be contaminated due to the physical admixtures, presence of pollen shedders and out crossing with foreign pollens. Hence, in seed industries, hybrid seed production has become dominant and hybrid seed purity is the most important aspect which is checked by

ICAR-Directorate of Onion and Garlic Research, Rajgurunagar-410 505, Pune (MS)
* Corresponding author's e-mail: guptaaj75@yahoo.co.in

grow-out-test (GOT). However, it is environment dependent and may vary the expression of specific morphological or physiological traits. Apart from that, major limitation factor of GOT will be the time and space required for assessing a large number of seed lots. To overcome this problem the molecular markers are being used in many of the crops. This made a way for use of molecular markers particularly the co-dominant markers. In recent years, Marker Assisted Selection (MAS) not only have been used for the analysis of genetic diversity but also for testing hybrid purity to overcome the shortcomings of morphology-based methods. A number of markers are being used to check genetic purity such as Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Sequence Related Amplified Polymorphism (SRAP), Inter Simple Sequence Repeat (ISSR), EST-SSR and SSR markers (Grzebelus et al. 2001, Ilbi 2003, Dongre and Parkhi 2005, Asif et al. 2009, Jadhav and Verma 2016). SSRs are locus-specific, co-dominant and unambiguous marker hence, widely used to assess hybrid purity, genetic fingerprinting and hybrid identification in onion (Mahajan et al. 2009, Saini et al. 2015), cauliflower (Zhao et al. 2012), maize (Hipi et al. 2013, Chaudhary et al. 2018), brinjal (Mangal et al. 2016) and vegetable crops (Padmanabha and Kiruthika 2018). The present study was done to confirm the hybridity of DOGR onion hybrids and to find the suitable SSR markers that can be utilize to distinguish the hybrids.

Materials and Methods

The samples used for hybridity test were taken from the experimental field of ICAR-Directorate of Onion and Garlic Research, Rajgurunagar, Pune, Maharashtra during 2017-18 and 2018-19. Total 16 onion genotypes including 9 hybrids and 7 parental lines were used for SSR profiling. The morphological features of the hybrids and parental lines were used in the present study are mentioned in Table 1.

DNA extraction: Total genomic DNA was extracted using CTAB method (Dellaporta et al. 1983). About 0.1 g of young leaf tissue from each sample was homogenized in liquid nitrogen and incubated at 65°C for 60 min with 500 ìl of CTAB buffer (1.0 M pH 8.0 Tris-HCl, 3 ml NaCl, 0.5 EDTA 10 µl â-mercaptoethanol). Then 500 ìl of chloroform: isoamyl alcohol mixture (24:1) was added and blended thoroughly. After centrifugation (5 minutes, 13,000 rpm) supernatant layer was pipette out into a new eppendorf tube and an approximately equal volume of chilled isopropanol was added. After storage at -20°C for 30–60 minutes, precipitated DNA was centrifuged and washed with ethanol and was air dried for half an hour and finally stored in sterile water. The DNA isolated was treated with RNase solution at 37 °C for 2 hours. The DNA quantification was done on 1% agarose gel stained with Ethidium Bromide and visualized in Gel Documentation system.

PCR amplification: Ten SSR primer pairs were used in the study (Table 2). The volume of the reaction mixture was 20 ìl which consists of 30 ng of template DNA, 10X PCR buffer without $MgCl_2$, 25 mM of $MgCl_2$, 10 mM of dNTPs, 0.25 ìM each of forward and reverse primers and 1U of Taq DNA polymerase (Thermo Scientific). The PCR program comprised an initial denaturation step at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 1 minute, annealing varies for the primers used between 50-55°C for 1 minute, extension at 72°C for 2 minutes and final extension at 72°C for 10 minutes. The SSR amplification products were separated on 7.5% non-denaturing

Table 1. Morphological characteristic of hybrids and parents used in the study

S. No.	Hybrid/ Parent	Parentage	Bulb Shape	Bulb Colour	Storability	Season
1	DOGR Hy-1	MS111A×Bhima Kiran	Flat Globe	Light Red	Good	*Rabi*
2	DOGR Hy-2	MS222A×Bhima Kiran	Globe	Dark Red	Good	*Rabi*
3	DOGR Hy-3	MS111A×Bhima Dark Red	Globe	Medium Red	Medium	*Kharif*
4	DOGR Hy-4	MS222A× Bhima Dark Red	Flat Globe	Dark Red	Medium	*Kharif*
5	DOGR Hy-5	MS1600A×Bhima Dark Red	Globe	Dark Red	Medium	*Kharif*
6	DOGR Hy-6	MS111A×DOGR1133	Flat Globe	Medium Red	Good	*Rabi*
7	DOGR Hy-7	MS222A×DOGR1133	Flat Globe	Dark Red	Very Good	*Kharif* and *Rabi*
8	DOGR Hy-8	MS1600A×DOGR1133	Flat Globe	Medium Red	Good	*Late Kharif*
9	DOGR Hy-50	MS1600A×Bhima Red	Globe	Dark Red	Very Good	*Rabi*
10	MS111A	-	Globe	Medium Red	Good	*Rabi*
11	MS222A	-	Flat Globe	Dark Red	Good	*Kharif*
12	MS1600A	-	Globe	Dark Red	Good	*Kharif*
13	Bhima Kiran	-	Globe	Light Red	Very Good	*Rabi*
14	DOGR1133	-	Globe	Light Red	Very Good	*Rabi*
15	Bhima Dark Red	-	Flat Globe	Dark Red	Medium	*Kharif*
16	Bhima Red	-	Flat Globe	Medium Red	Medium	*Kharif and Rabi*

Table 2. Details of SSR markers used for *Allium* genotypes evaluation

S. No.	Marker	Forward and Reverse Primer Sequence (5'–3')	Tm	Alleles	He	PIC value	Polymorphism status
1	ACM008	F- GCCGGAAGAGGAGAAGAAGT R- CATAATTCCCATGGCTTTGC	50.3	4	0.6200	0.5490	Monomorphic
2	ACM080	F- GCATTATGCAGTAACGGGCT R- GCAGCAGCATTTGATTGAAC	50.3	2	0.7244	0.6865	Monomorphic
3	ACM093	F- GCCAACAGTTTTCGTAAGTTGA R- ATTCTCTTCGGCTTTCGTGA	50.3	2	0.7486	0.7137	Polymorphic
4	ACM018	F- GGGGAATGGTGGAGAATAGA R- AACAGAGGCAAGAGGAGCG	52.3	4	0.6639	0.6001	Monomorphic
5	ACM033	F- CCTTCTCCCCATTCTCTTCC R- ATCATCGTCCTCGTCCTCAT	52.3	3	0.6593	0.5949	Polymorphic
6	ACM034	F- CACCTTGGACCGTGAAGAAC R- CTGCTGTTTGGAGATGTGGA	52.3	3	0.5067	0.4323	Monomorphic
7	ACM038	F- ATGCCAGACTACGACAACGA R- ACGCCTACCAACCTTCAATG	52.3	3	0.6811	0.6166	Monomorphic
8	ACM180	F- CCTTCAGACCCTAAAAGGGC R- CAAAGGACATTGGCAAGTGA	50.3	3	0.546	0.4986	Monomorphic
9	ACM078	F- CGCAGAATCTCGTCCTTTTT R- AATGGTTTGGAGGTCAGTCG	50.3	2	0.7111	0.6628	Polymorphic
10	ACM016	F- ATGGAAGCCTCGGGTCTG R- GCCGTAAGTCGAGGGTAGAA	53.2	2	0.6224	0.5874	Polymorphic
	Total			**28**	**6.485**	**5.9419**	
	Average			**2.8**	**0.6485**	**0.59419**	

Tm = Annealing temperature, He = Expected heterozygosity, PIC = Polymorphism Information Content

polyacrylamide gels and detected by staining with EtBr at 100-120 V/cm for 100-200 minutes and photographed using gel documentation unit under UV light.

Results and Discussion

Genetic purity testing of hybrids is important for successful varietal improvement, release and seed production programme. Unique, clear and distinct patterns can be obtained using the molecular markers for the identification and characterization of cultivars. Use of DNA marker is advantageous over morphological and biochemical markers. The morphological markers are influenced by the environmental conditions, labour intensive, time consuming and difficult to perform. Biochemical markers such as isozyme and protein profiling are least influenced by the environment, but they exhibit less polymorphism and do not differentiate between closely related inbred lines (Pallavi et al. 2011).

Ten SSR markers were screened to distinguish the parental lines. A total of 28 alleles were obtained from 10 SSR markers with an average of 2.8 alleles per primer. The number of alleles amplified for each primer pair ranged from 2 to 4 and the PIC value ranged from 0.4323 to 0.7137 and heterozygosity from 0.5067 to 0.7486 (Table 2). The marker ACM008 and ACM018 amplified a maximum four alleles, while four SSR markers (ACM033, ACM034, ACM038 and ACM180) amplified three alleles each. Two alleles were amplified by the remaining four markers (ACM016, ACM078, ACM080 and ACM093). It was seen that among the ten SSR markers, six primers were identified as monomorphic (ACM008, ACM018, ACM034, ACM038, ACM080 and ACM180) (Fig. 1), while the rest of four primers *viz*., ACM016, ACM033, ACM078 and ACM093 were found to be polymorphic (Fig. 2). Three markers ACM016, ACM033 and ACM078 having PIC value 0.5874, 0.5949 and 0.6628 and expected heterozygosity value 0.6224, 0.6593 and 0.7111, respectively are potential to differentiate each parent of hybrids. Thus, SSR markers can be successfully applied to distinguish and identify the hybrids from its parental lines.

The study was undertaken to confirm the hybridity of *Allium cepa* L. based on the amplification pattern of SSR markers. The complementary banding pattern of the male and female parents helped to confirm the genuineness of F_1 plants developed. The bulbs of two genotypes used as female parent and male parent, were grown in field condition and crossed to develop F_1 seeds. About 10 random SSR primers were used for screening of parental genotypes for polymorphism. The primers associated with each hybrid and parental lines were assessed on 3% agarose. Since the PCR products of some sample failed to show any polymorphism among the hybrid and their parental lines on agarose therefore, polyacrylamide gel electrophoresis was used for resolving the bands. Based on the complementary banding patterns between the hybrid plants and parents, the polymorphic SSR markers ACM016, ACM033 and

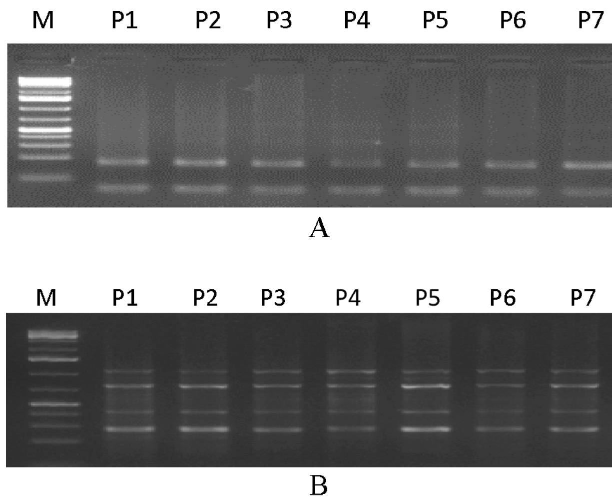

Fig. 1. Monomorphic banding pattern of SSR primers with parental genotype with marker A: ACM080 and B: ACM018 (P1: MS111A, P2: MS222A, P3: MS1600A, P4: Bhima Kiran, P5: DOGR1133, P6: Bhima Dark Red and P7: Bhima Red)

ACM078 were identified as the specific markers which enable to distinguish and identify hybrid from their parental lines. As anticipated same alleles were detected in a hybrid when the parents were monomorphic for a particular SSR locus and two to three alleles were in hybrid when polymorphism was detected between the female parental and male sterile lines. Daniel et al. (2012) in maize and Mangal et al. (2016) in brinjal showed that SSR markers were remarkably reliable for identifying genetic purity as compared to the morphological markers.

Confirmation of hybridity: Attempts were made to identify SSR markers which could be used to confirm hybridity of nine hybrids. The three selective markers were used to confirm hybridity of nine hybrids from seven parental lines. The profile of ACM016 marker amplified four alleles of size 600 bp, 400 bp, 380 bp and 210 bp in its male fertile line (Bhima Kiran) and a single allele of size 210 bp in its male sterile line (MS111A). Hence, DOGR Hy-1 showed the expression of both the alleles of male fertile and male sterile line which showed presence of hybridity.

The marker amplified four alleles of size 600 bp, 400 bp, 380 bp and 210 bp in male fertile line (Bhima Kiran) whereas in male sterile line (MS222A) two alleles of size 210 bp and 200 bp. This confirmed the crossing and hybridity between two parents in DOGR Hy-7 using ACM016. In DOGR Hy-8 the same marker was able to distinguish heterozygosity in male and female parental lines. The banding pattern of four alleles were seen in male fertile line (DOGR1133) of size 600 bp, 400 bp, 380 bp and 210 bp and single allele of size 210 bp in male sterile line (MS1600A). This showed the complementary bands in DOGR Hy-8, thus confirming hybridity. The profile of ACM033 marker amplified three alleles in male sterile line (MS222A) of size 280 bp, 190 bp and 180 bp whereas in male fertile line (Bhima Kiran) three alleles were identified with size 310 bp, 280 bp and 190 bp. However, both male and female alleles were expressed in DOGR Hy-2. Although in Fig. 3 (A) several bands are visible, only those bands having presence of alternate alleles in male and female parents were considered for hybridity.

The profiling of same marker ACM033 amplified two alleles of size 280 bp and 180 bp in male sterile line (MS111A) whereas in male fertile line (Bhima Dark Red), it showed the presence of three alleles of size 300 bp, 280 bp and 180 bp. The expression of complement bands of both male and female parents was recognized in DOGR Hy-3 (Fig. 3 B). The marker amplified 3 alleles of size 280 bp, 250 bp and 180 bp in male fertile line (Bhima Dark Red) and two alleles of size 250 bp and 190 bp in male sterile line (MS222A). The complement banding pattern was observed in DOGR Hy-4 which confirmed the hybridity using ACM033 marker.

The profile of marker ACM033 was found to be polymorphic in a cross between MS1600A and Bhima Dark Red which amplified three alleles in male sterile parent with size 250 bp, 190 bp and 180 bp whereas three alleles in male fertile line of size 300 bp, 250 bp and 190 bp. The band obtained in DOGR Hy-5 showed

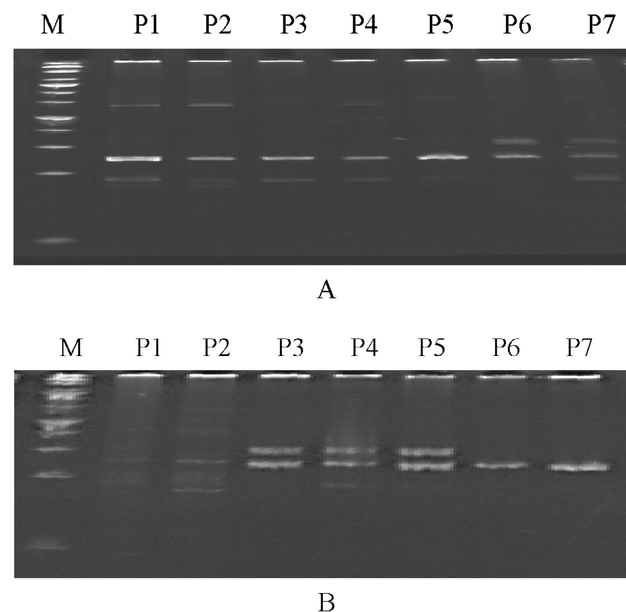

Fig. 2. Polymorphic banding pattern of SSR primers with parental genotypes with marker A: ACM033 and B: ACM078 (P1: MS111A, P2: MS222A, P3: MS1600A, P4: Bhima Kiran, P5: DOGR1133, P6: Bhima Dark Red and P7: Bhima Red)

the presence of both male sterile line and male fertile line thus confirming hybridity (Fig. 3 C). Similar findings were reported by Ye et al. (2013) in cabbage and Pattanaik et al. (2018) in cauliflower. The profile of ACM078 showed the expression of both male and female parental alleles as a result of crossing between two parents in DOGR Hy-6. The marker amplified single allele of size 250 bp in male sterile line (MS111A) whereas in male fertile line (DOGR1133), three alleles of size 300 bp, 250 bp and 180 bp were identified (Fig. 3E). This confirmed the crossing and hybridity between two parents. Similar results were observed in tomato by Paran et al. (1995) and in brinjal by Kumar et al. (2014).

The banding pattern using ACM033 marker in DOGR Hy-50 showed the presence of three alleles in male sterile parent and four alleles in female parental line with size 250 bp, 190 bp and 180 bp in male sterile line (MS1600A) and 300 bp, 250 bp, 190 bp and 180 bp in female parent (Bhima Red). The hybrids showed the presence of complement bands of both male and female parent in the resultant crossing in DOGR Hy-50 (Fig. 3 D). Results of the present investigation are in agreement with the conclusion of Daniel et al. (2012) in maize. Cytoplasmic male sterility system is efficient for use in hybrid seed production as it eliminates the need of hand emasculation. The hybrids developed at DOGR using CMS lines showed high percentage of heterosis over the standard checks (Gupta and Singh 2016). All the hybrids developed showed good storability, early maturity and were free from doubles and bolters (Gupta et al. 2018).

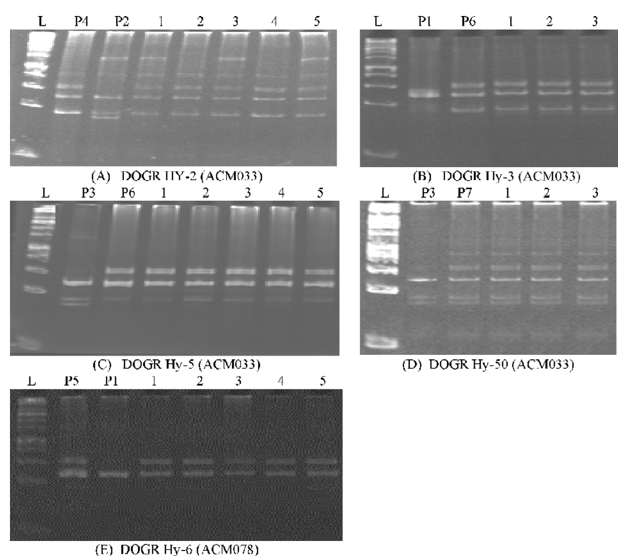

Fig. 3. SSR profiling of ACM033 and ACM078 obtained on Ethidium Bromide stained PAGE (7.5%) Lane M=100 bp ladder; P1: MS111A, P2: MS222A, P3: MS1600A, P4: Bhima Kiran, P5: DOGR1133, P6: Bhima Dark Red and P7: Bhima Red

Note: 1, 2, 3, 4 & 5 = DOGR Hybrid Samples in case of DOGR Hy-2, DOGR Hy-5 and DOGR Hy-6; 1, 2 & 3 = DOGR Hybrid Samples in case of DOGR Hy-3 and DOGR Hy-50

The present investigation showed that SSR markers are quick, reliable, robust and effective for assessing genetic purity as compared to morphological and biochemical markers. The SSR primers identified in the study can be utilize for routine genetic purity testing of DOGR hybrids of onion. The information generated through SSR markers will be immensely helpful for hybrid identification in onion.

सारांश

प्याज की नौ संकर किस्मों एवं उनके जनकों के आण्विक पहचान और संकर शुद्धता परीक्षण के लिए आईसीएआर–प्याज एवं लहसुन अनुसंधान निदेशालय, राजगुरू नगर, पुणे (महाराष्ट्र) में परीक्षण किया गया। दस माइक्रोसेटेलाइट (एस.एस.आर.एस–सिंपल सीकक्स रिपीट) मार्करों में से 4 पालीमॉर्फिक पाए गये। सभी चार बहुरूपी मार्करों ने एक या अधिक संकरों के नर और मादा जनक के बीज बहुरूपता का पता लगाया गया और उनके संकर परीक्षण के लिए उपयोग किया गया। ए.सी.एम.ओ.–16, ए.सी.एम.ओ.–33 और ए.सी.एम.ओ.–78 को इस अध्ययन में शामिल सभी नौ प्याज संकरों (डी.ओजी.आर. हाइब्रिड–1, डी.ओजी.आर. हाइब्रिड–2, डी.ओजी.आर. हाइब्रिड–7, डी.ओजी.आर. हाइब्रिड–8 और डी.ओजी.आर. हाइब्रिड–50) की आनुवंशिक शुद्धता के परीक्षण के लिए पहचाना गया जो 3 नैदानिक मार्कर के रूप में पाया गया। इन 10 एस.एस.आर. लोसायी ने कुल 28 एलील एम्प्लिफिएड किये जो 9 संकरों और 7 जनकों में प्रति लोसायी 2–4 एलील थे। औसतन 2.8 एलील प्रति लोसायी पाई गयी। सबसे अधिक बहुरूपता सूचना सामग्री (पी.आई.सी. मान) मार्कर ए.सी.एम.ओ.–93 के लिए 0.7137 और तीन संभावित मार्कर ए.सी.एम.ओ.–16, ए.सी.एम.ओ.–33 और ए.सी.एम.ओ.–78 के लिए पी.आई.सी. मान क्रमशः 0.5874, 0.5949 और 0.6628 के साथ देखे गये जो सबसे अच्छे मार्कर थे तथा पित्र को अलग करने और संकर परीक्षण के लिए उपयोग किया जा सकता सकता है। इस अध्ययन से पता चला कि एस.एस.आर विश्वसनीय और प्रभावी आण्विक मार्कक हैं जो संकर प्याज की अनुवांशिक शुद्धता का पता लगाने में सक्षम है।

References

Asif M, Rahman MU, Mirza JI and Zafar Y (2009) Parentage confirmation of cotton hybrids using molecular markers. Pak J Bot 41: 695-701.

Barham WS and Munger HM (1950) The stability of male sterility in onions. J Amer Soc Hort Sci. 56: 401–409

Chaudhary S, Yashveer S, Dinesh, Prajapati D R, Kharb P and Kamboj M R (2018) Utilization of SSR markers for seed purity testing in popular maize hybrids. Int J Curr Microbiol App Sci 7: 1117-1126

Daniel IO, Adetumbi JA, Oyelakin OO, Olakojo SA, Ajala MO and Onagbesan SO (2012) Application of SSR markers for genetic purity analysis of parental inbred lines and some commercial hybrid maize (*Zea mays* L.). American J Experimental Agri 2: 597.

Dellaporta SL, Wood J and Hicks JB (1983) A plant DNA mini preparation: Version II. Plant Mol Bio Reporter 1: 19-21.

Dongre A and Parkhi V (2005) Identification of cotton hybrid through the combination of PCR based RAPD, ISSR and microsatellite markers. J Plant Biochem Biotech 14: 53-55.

FAOSTAT (2018) Food and Agriculture Organization (FAO) of the United Nations Statistics Division. Economic and Social Development Department, Rome, Italy. Available at http://www.fao.org/faostat.

Grzebelus D, Senalik D, Jagosz B, Simon PW and Michalik B (2001) The use of AFLP markers for the identification of carrot breeding lines and F_1 hybrids. Plant Breeding 120: 526-528.

Gupta AJ and Singh BK (2016) Development of hybrids and hybrid seed production in onion. Training Manual on "Principles and Production Techniques of Hybrid Seeds in Vegetables", organized by ICAR-IIVR, Varanasi, pp. 101-111.

Gupta AJ, Mahajan V, Lawande KE and Singh M (2018) Evaluation of F_1 hybrids in onion developed through male sterility. J Agri Search 5: 163-168.

Hipi A, Surahman M and Ilyas S (2013) Seed genetic purity assessment of maize hybrid using microsatellite markers (SSR). Int J Appl Sci Tech 3: 66-71.

Ilbi H (2003) RAPD markers assisted varietal identification and genetic purity test in pepper, *Capsicum annuum*. Scientia Hort 97: 211-218.

Jadhav V and Verma OP (2016) Assessment of genetic purity in hybrid lines by field grow-out test and molecular markers. Asian J Bio Science. 11: 106-118

Jones HA and Clarke A (1943) Inheritance of male sterility in the onion and the production of hybrid seed. J Amer Soc Hort Sci 43: 189-194.

Jones HA and Emsweller S (1936) A male-sterile onion. J Amer Soc Hort Sci 34: 582-585.

Kumar AMB, Dadlania M, Kumar R, Jacob SR (2014) Identification and validation of informative SSR markers suitable for ensuring the genetic purity of brinjal (*Solanum melongena* L.) hybrid seeds. Scientia Hort 171: 95–100

Mahajan V, Jakse J, Havey MJ and Lawande KE (2009) Genetic fingerprinting of onion cultivars using SSR markers. Indian J Hort 66: 62-68.

Mangal M, Upadhyay P and Kalia P (2016) Characterization of cultivated and wild genotypes of brinjal (*Solanum melongena* l.) and confirmation of hybridity using microsatellite markers. Vegetos-An Int J Plant Res 29: 27-34.

Padmanabha BV and Kiruthika S (2018) Identification of off-type in F_1 hybrids of commercially cultivated vegetable crops using SSR markers as a molecular tool. International Journal for Research in Applied Science & Engineering Technology. 6: 2790-2796

Paran I, Horowitz M, Zamir D and Wolf S (1995) Random amplified polymorphic DNA markers are useful for purity determination of tomato hybrids. Hort Science 30: 377.

Pattanaik A, Lakshmana Reddy DC, Ramesh S and Chennareddy A (2018) Comparison of traditional grow-out test and DNA-based PCR assay to estimate F_1 hybrid purity in cauliflower. Current Sci 115(11): 2095-2102.

Saini N, Hedau NK, Khar A, Yadav S, Bhatt JC and Agrawal PK (2015) Successful deployment of marker assisted selection (MAS) for inbred and hybrid development in long-day onion (*Allium cepa* L.). Indian J Genet Plant Breed 75(1): 93-98.

Williams IH and Free JB (1974) The pollination of onion (*Allium cepa* L.) to produce hybrid seed. J Appl Eco 11: 409-417.

Ye Shan, Wang Y, Huang D, Li J, Gong Y, Xu L and Liu L (2013) Genetic purity testing of F_1 hybrid seed with molecular markers in cabbage (*Brassica oleracea* var. *capitata*). Scientia Hort 155: 92-96.

Zhao Z, Gu H, Sheng X, Yu H, Wang J and Cao J (2012) Genetic purity testing of loose-curd cauliflower hybrids using SSR markers and grow out test. Seed Science and Technology, 40: 209-214.