Environment and Ecology 40 (4B) : 2359—2363, October–December 2022
ISSN 0970-0420

A Review on Genetic Purity Assessment of Seeds using Molecular Markers

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Received 28 July 2022, Accepted 22 September 2022, Published 10 November 2022

ABSTRACT

Genetic purity testing and varietal verification are very important aspects in quality seed production. Genetic purity is referred as the free from contaminations as other variety seeds in the original variety. Generally the grow out test is been used for testing the genetic purity of seed lots of different classes of the seed. Recently, several issues regarding the genetic deterioration of different varieties are noticed. This may be due to environmental variations or may be due to inappropriately checking the purity of the seed lots. The grow out test is very tedious, need a dedicated expert for doing the test and it also consumes space and time. In order to find a quick and alternate methods to GOT the molecular markers are employed for several crops. Generally GOT is based on morphological characters and it is highly difficult to find the differences between the varieties which are having similar morphological character so it become inevitable to go for marker in such conditions. Being objective, efficient, time-saving, less labor intensive and reproducible, the molecular markers would play an important role in cultivar identification and genetic purity testing of seed.

Keywords Grow out test, SSR markers, SNP markers, Genetic purity testing, Chemical /Biochemical methods.

INTRODUCTION

Now days it is becoming compulsory to conduct GOT test for breeder seed also. GOT is a tedious process, need a dedicated expert for doing the test and it also consumes space and time. Genetic purity contaminations may occur due to pollen shedders and physical admixtures during processing. GOT require full growing season, land, labor, tedious and it is subjected to human and environmental errors while detecting the off types. During early 90s biochemical markers viz., isozymes and IEF technologies were also employed earlier as an alternative to GOT. From recent past the DNA markers viz., RFLP, AFLP, RAPD, SSR, ISSR and SNPs were also employed for genetic purity testing and among these the most widely used ones are SSRs and SNPs. To detect loci in parental inbred lines and corresponding F_1s is the most important step in seed genetic purity testing of F_1 hybrid.

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Even though there are strict DUS guidelines are there several recently released varieties look morphologically similar making it difficult to differentiate. Molecular markers are playing an important role in resolving several such complications. The degree of contamination due to selfing and out crossing in hybrid seed lot and obtaining a specific sequence pattern for each variety are essential in seed technology. The type of marker to use will depend on the crop we are dealing with as well as reproductive behavior of crop. The bi-parental nuclear, co-dominant markers used for identification of selfed female seed in hybrid seed lot, identification of isogenic B line contamination in female A line (parental purity testing) in hybrid seed production. For trait purity testing or trait confirmation we use maternal nuclear and dominant markers. Owing to the low genetic variation in self pollinated crops researchers use dominant markers like RAPD and AFLP for detecting the polymorphism in self pollinated crops. Certain times dominant markers were also used for detection where there is no sequence information is available. Molecular markers can be employed in the system of genetic purity and varietal verification in order to strengthen the seed sector. Despite, there are certain issues like standardization of seed sample size for molecular methods, sampling grow-out matrix for validity of molecular results and guidelines for use of SSR markers and SNP markers in genetic purity testing can be future line of work. The molecular markers would be more efficient than GOT, since DNA markers would be more accurate for determining hybrid seed purity. Further, marker analysis will also result in considerable savings for the seed industry as this technique may avoid the cost of storage for a whole season and cost of acquiring land and cultivation.

SSR markers in genetic purity assessment in crop plants

Kumar *et al.* (2021) used 55 SSR markers for identification of genetic impurities in 16 high yield varieties of rice. 14 primer pairs were found to be polymorphic and amplified 48 alleles with an average of 3.43 alleles per each primer pair. The number of alleles amplified ranged from 2 to 6 and the size of the PCR products amplified from these 14 primer pairs ranged from 80 to 450 bp with polymorphic information content (PIC) from 0.14 (RM 346) to 0.99 (RM 5900) and confirmed that the SSR markers are highly informative and could be potentially used for distinctness, uniformity and stability (DUS) testing and genetic purity analysis.

Nagendra *et al*. (2021) used grow out test as well as molecular markers (SSR) for assessing the genetic purity in paddy. They found that SSR marker RM 570 can differentiate the CMS 23A and CB 174 R lines with the allelic size of 260 and 300 bp respectively. Four hundred paddy seeds comprising 2 off type and 398 original seeds of A, R and CO RH 4 were sown in 20 rows × 20 columns matrix in a sand medium and leaf samples from seedlings were bulked row and column wise. RM 570 produced single allele for genetically pure bulking and two alleles for bulking with off types. Further, the identified off-types were investigated by individual leaf analysis using RM 570 marker.

Rakesh *et al*. (2019) used forty three simple sequence repeat (SSR) markers to distinguish ten pigeon pea varieties (Maruti, TS3R, GRG-811, Asha, BSMR-736, GRG833, GC-11-39, WRP-1, ICPL-87 and Bannur local) with an average of 2.81 alleles per marker and found that sixteen markers were polymorphic to ten varieties having PIC values ranging from 0.567 to 0.695. The variety specific markers viz.,CcM0039 (GC-11-39), CcM0047 (GRG-833), CcM0057 (Maruti), CcM0082 (GRG-811), CcM0093 (Bannur Local), CcM0095 (GC-11-39), CcM0133 (Maruti), CcM0185 (WRP-1), CcM0193 (GRG-833), CcM0248 (WRP-1), CcM0252 (ICPL-87), CcM0257 (ICPL-87), CcM0271 (BSMR-736), CcM0293 (TS3-R), AHSSR46 and AHSSR48 (Asha) were identified and concluded that genetic purity testing using SSR markers is more efficient and faster compared to field grow out test and chemical methods.

Xia *et al.* (2018) employed fifty-five sets of SSR markers and identified fourteen markers for assessing hybrid purity of breeding lines. Cross-checking assay validated nine SSR markers as informative SSR markers for purity detection of these hybrids. SSR markers ClSSR09643 and ClSSR18153 were used for purity detection of two hybrids. Genetic purity of six commercial watermelon hybrids was evalu-

ated using these SSR markers. Genetic purity of all tested hybrids exceeded 96% while the field purity was above 98%.

Shalu *et al*. (2018) used microsatellite markers for fingerprinting of hybrids, for assessing variation within parental lines and testing the genetic purity of hybrid seed lot. 75 simple sequence repeat (SSR) markers well distributed on all the 10 chromosomes were employed for fingerprinting of six popular maize hybrids and their parental lines of which seven SSR markers were found polymorphic and produced unique fingerprints for the different hybrids and they can be used as referral markers for unambiguous identification and protection of the hybrids.

Anjana *et al.* (2016) made an attempt to find out an alternative to Grow-Out-Test, which is very tedious, time consuming and used conventionally for seed genetic purity testing since beginning of quality seed multiplication chain. Fifty-one rice-specific sequence tagged microsatellite (STMS) primer pairs distributed throughout the rice genome were employed for fingerprinting of eight rice hybrids and their parental lines with a view to assess variation within parental lines to test the genetic purity of the commercial seed lots. Out of 51 markers, 28 microsatellite markers showed polymorphism. A total of 98 alleles were obtained with an average of 1.92 alleles per primer pair and number of alleles amplified for each primer pair ranged from 1 to 4.

Lakshmana Reddy *et al.* (2015) tested hybrid purity of F_1 hybrids through grow-out tests, and molecular markers. They assessed the purity of inter specific hybrids of *Solanum melongena* L. ('IIHR3', Arka Keshav ('AK'), '2BMG') and *Solanum macrocarpon* L. ('SM') using simple sequence repeats (SSR). Genomic DNA from parents and F_1 hybrids were subjected to SSR analysis to detect parental polymorphism. Among 119 SSRs screened, 5 SSRs were co-dominant. There were five unique microsatellite markers, two for IIHR3 × SM, emf 01C03 and emh 02E08, one for AK ×SM, emi 02 F16; and two for 2BMG ×SM, emb 01E 03 and emg 11 I03, were useful to detect purity of three inter specific eggplant hybrids.

Zhang *et al.* (2014) screened 5 parental lines of soybean using 160 Simple Sequence Repeat markers of which 8 markers exhibited polymorphism. They confirmed that for detection of contaminations in hybrids from their parents a single polymorphic marker was sufficient. It was also found that if a hybrid seed lot was contaminated by another hybrid or its parental lines and determined this method could accurately and effectively identify the hybrid purity in a predetermined sample of soybean hybrids constituted by deliberately mixing seeds of parental lines.

Sudharani *et al.* (2014) investigated to identify specific marker associated with the maize hybrid DMH-117 and its parental lines, hybrid purity testing as well as identify the off-types from the seed lot. The hybrid DHM-117 could be clearly identified by using the SSR loci Umc1600 based on the banding pattern resolved on agarose gel. The complementary banding pattern of the male and female parents made a way to identify the hybrid. The SSR loci Umc1600 amplified a specific allele size of 180 bp in seed parent (BML-6) and 195 bp was specific to pollen parent BML 7. These two bands of allele size 180 bp and 195 bp were found in hybrid DHM-117, there by confirming that this hybrid was produced from the cross combination of BML-6 and BML 7.

Daniel *et al.* (2012) used six SSR markers for estimating the genetic distance among the inbred lines and hybrids in maize. Genetic purity of each genotype were calculated from the standard genetic distance indices and the results inferred that the inbred parents of Oba Super 1 (1368 and 9007) recorded higher purity of 97.3% and 98.7% while inbred parents of Oba Super 2 (4001 and KU-1414SR) showed purity of 96% and 91.3% respectively which clearly indicated the fact that the purity level of the inbred parents will determines the purity of the hybrids because Oba Super 1 has higher purity level than Oba Super 2.

Pallavi *et al.* (2011) investigated to identify pure hybrid and pollen shedders/off types by using SSR marker in sunflower. 58 primer pairs were screened to identify the specific marker associated with each hybrid and parental lines. Hybrid KBSH-44 could be clearly identified by using ORS 309 and ORS 170, based on the banding pattern resolved on polyacrylamide gel (6%). The complementary banding pattern

of both parents made a way to identify the hybrid. ORS 309 amplified allele size at 250 bp was specific to female parent (CMS-17A) and 230 bp was specific to male parent (RHA 95- C-1). These two bands of allele size 230 and 250 bp were found only in hybrid KBSH-44. Another SSR primer ORS 170 was able to distinguish the hybrid KBSH-44 by amplifying allele of size 230 bp a female specific (CMS-17A) allele and 200 bp amplicon a male specific allele (RHA 95-C-1). SSR primer ORS 811 found specific to identify KBSH-53 and it amplified allele of size 270 bp in its female parent (CMS-53A) and allele size of 230 bp in its pollen parent (RHA 95-C-1). The hybrid has both the alleles from its parents at 270 and 230 bp.

Selva kumar *et al.* (2010) conducted the genetic purity assessment of three cotton hybrids TCHB 4510, TCHB 2310 and TCHB 213 by GOT and molecular markers. 400 individual's plants from each one of the three hybrids were raised in the field and morphological traits were recorded. GOT results showed that TCHB 2310 recorded lowest genetic purity (62.5%) followed by TCHB 4510 (78.2%) and TCHB 213 (95.2%). SSR marker analysis of parents involved in the production of all the three hybrids have shown that 45 out of 150 SSRs were polymorphic among the parents. The SSRs, BNL686, BNL1679, BNL3971, BNL3955, CIR407 and CIR413 were selected to test the genetic purity of hybrid seeds since they have produced clear, scorable and unambiguous polymorphic bands among the parents.

Josia *et al.* (2021) studied genetic purity and diversity among the inbred lines using 92 single-nucleotide polymorphism (SNP) markers. A total of 188 maize genotypes, comprising of 26 inbred lines, four doubled haploid (DH) lines and 158 single-cross maize hybrids were investigated using Kompetitive Allele Specific Polymerase Chain Reaction (KASP) genotyping assays. The bi-allelic data was analyzed for genetic purity and diversity parameters using Gen Alex software.

The SNP markers found highly polymorphic and 90% had polymorphic information content (PIC) values of > 0.3. The majority (67%) of the inbred lines studied were genetically pure with residual heterozygosity of 5%. Inbred lines, which were not pure, require purification through further inbreeding. Out of the 68 hybrids (43%) that passed the parent-offspring test, seven hybrids namely; SCHP29, SCHP95, SCHP94, SCHP134, SCHP44, SCHP114 and SCHP126, were selected as potential candidates for further evaluation and release due to their good yield performance. Significant changes in the genetic constitution of the inbred lines affect both the quality of newly developed hybrids and hybrid seed. The routine quality control genotyping in this study was done to detect any contamination which could have happened during hybrid development and inbred lines maintenance. The 14 inbred lines (CB323, CML202, CML216, CML442, CML443, CML511, CML544, CZL 068, I-38, 1-42, CML 540, CZL99017, CML312 and I-40) with 100% genetic purity were generated through conventional system. This affirms that the maintenance of these inbred lines was carefully done for several generations of selfing for seed increase and they are suitable for high quality hybrid production. Similar findings were reported by (Dao 2013) in his study using 1237 SNPs, where the majority of the inbred lines tested exhibited 100% homozygosity. Ertiro *et al.* (2017) reported that of the inbred lines tested using 22,787 SNPs, only 22% of the inbred lines had 99.9% genetic purity.

CONCLUSION

The molecular markers are more efficient than GOT, since DNA markers would be more accurate for determining hybrid seed purity. Further, marker analysis will also result in considerable savings for the seed industry as this technique may avoid the cost of storage for a whole season and cost of acquiring land and cultivation.

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