*International Research Journal of Pure & Applied Chemistry*

***21(22): 31-40, 2020; Article no.IRJPAC.62758***
***ISSN: 2231-3443, NLM ID: 101647669***

# Application of SSR Markers for Purity Testing of Hybrid Wheat (*Triticum aestivum* L.)

**Akanksha Tiwari[1*] and D. K. Mishra[2]**

*[1]ANDUAT, College of Agriculture, Azamgarh (U.P.)-276001, India.*
*[2]JNKVV, Jabalpur (M.P.), India.*

***Authors' contributions***

*This work was carried out in collaboration between both authors. Author AT performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript managed the analyses of the study, managed the literature searches. Author DKM designed the study. Both authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/IRJPAC/2020/v21i2230294
Editor(s):
(1) Dr. Hao-Yang Wang, Shanghai Institute of Organic Chemistry, China.
Reviewers:
(1) Md. Abubakar Siddique, Bangladesh Rice Research Institute, Bangladesh.
(2) Alvin D. Palanog, Philippine Rice Research Institute, Philippines.
Complete Peer review History: http://www.sdiarticle4.com/review-history/62758

***Original Research Article***

***Received 05 September 2020***
***Accepted 11 November 2020***
***Published 30 November 2020***

## ABSTRACT

Exploitation of the full potential of any hybrid requires the possessing of genetically high-purity seeds. In order to avoid reduction in yield caused by using low purity seeds, development of a simple, rapid, and accurate method for hybrid purity assessment is of great essence and significance. For the identification of true hybrids, SSR markers provides authentic information that will help to the further progress of any research programme. In this study, total of 40 wheat hybrids and 14 parental lines were taken for the hybrid purity analysis with 20 SSR markers. These markers were used to find out the codominant loci in the hybrid and single dominant loci in parents. Out of 20 SSR markers, only 8 markers viz., Xwmc617, Xwmc457, Xwmc48, Xgwm153, Xbarc61, Xgwm273, Xbarc268 and Xgwm274 showed the polymorphic dominant loci in the parents and co-dominant loci midway between these parents. Therefore these SSR markers were used to confirm the forty hybrids. The range of allele size produced by SSR marker on the parental lines were 120 bp to 300 bp. The highest allel size (300 bp) was produced by marker Xbarc 268 whereas, the minimum allel size (120 bp) was produced by marker Xgwm 273. All the fourty hybrids showed similar banding pattern as parental lines, this proved the purity of hybrids in all these cross combinations. The confirmation of hybrid purity indicated that a single polymorphic marker was sufficient for detection of contaminations of these hybrids from their parents.

**Corresponding author: E-mail: twri.akanksha@gmail.com;*

*Keywords: Wheat hybrids; SSR marker; codominant; polymorphic.*

## 1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is the first important and strategic cereal crop for the majority of world's populations. It is the most important staple food of about two billion people (36% of the world population). Wheat belongs to the tribe *Triticeae*(= *Hordeae*) in the grass family *Poaceae*(*Gramineae*). Initially available wheat genome (2n = 6x = 42) is huge (~17 GB), complex (AABBDD, 3 homoeologous subgenomes), with high repeat content (80%) which was having more than 124 thousand genes [1] Wheat is the second most important grain crop after rice in India and has tremendous yield potential. Currently India is second largest producer of wheat in the world after china which contributed 14% share in total world wheat production. Increasing winter wheat (*Triticum aestivum* L.) grain yield and developing cultivars better adapted to climactic variability is crucial for agricultural productivity and food security. Unlike other cereals, wheat contains a high amount of gluten, the protein that provides the elasticity necessary for excellent bread making. Developing hybrid wheat may be a way to address these goals Gowda et al., [2] Higher genetic purity is an essential pre–requisite for commercialization of any hybrid. Further, identification of hybrids, parental lines and determination of their genetic relatedness are very important for variety registration system, DUS testing and Protection of Plant variety and Breeder's rights. So, clear cut identification of elite crop varieties/hybrids and corresponding parental lines is essential for protection and prevention of unauthorized commercial usage. Globally there are large number of varieties and such varietal differentiation also requires whole genome scanning of SSRs in order to get polymorphic markers. Molecular markers are also required for wheat seed purity testing and differentiation of hybrids [3]. SSR markers can be used in varietal differentiation and traceability of the wheat produce and products [4].

In wheat, DNA markers represent an exciting new tool for studying genetic relationships between species, populations and cultivars. For this purpose different marker types have been used with varying results. Assessment of genetic purity of hybrid seed based on fertility restorer gene linked to co-dominant STMS marker was found to be more reliable than the non linked one [5]. Group of Indian wheat varieties/AVT lines have been differentiated using limited set of markers [6]. Such use of SSR profiling in uniformity testing to confirm DUS test of variety has already been reported in wheat [7].

Simple sequence repeat (SSR), has been developed based on repeated DNA sequence variation and applied in many cereal crops including rice, maize and barley. In common wheat, which has a characteristic of large genomes and allopolyploidy, SSR is one of the most useful markers. The marker has the advantage of being genome-specific, codominant, and multiallelic, thus is suitable for mapping important genes in wheat [8]. Presence or absence of any particular band helps in demarcation and identification of variety [9]. Wheat microsatellites have been successfully used for the construction of genetic linkage maps of wheat, for detection of genetic diversity and for mapping of agronomicaly important genes. These markers are also an efficient tool for hybrid authentication.

Therefore, the present investigation was conducted with the purpose of finding out a specific SSR marker to distinguish wheat hybrids from its parental lines.

## 2. MATERIALS AND METHODS

### 2.1 DNA Isolation

Genomic DNA was isolated using the CTAB method [10] with minor modifications. In our study 1.8% CTAB was used in DNA extraction buffer for DNA isolation from young leaves of 10 plants of each parental and hybrid line. The quantity and quality of DNA was assessed with 0.8% agarose gel electrophoresis referring to a series of standards of known quantities.

### 2.2 Chemicals

The chemicals used in the investigation were of molecular biology grade or analytical grade of Sigma chemicals Co. USA, Merck Pvt. Ltd. India.

### 2.3 DNA Extraction Buffer

The buffer was prepared as per specification given in Table 1.

## 2.4 Sources of SSR Markers

A total of 20 SSR primers comprising 4 Xbarc [11], 6 Xgwm [8], 10 Xwmc [12], distributed across different wheat chromosomes were used in this study (Table 2).

**Table 1. Composition of DNA extraction buffer**

| Sr no | Chemicals | Final concentration | Working concentration |
|---|---|---|---|
| 1 | Tris HCL (Ph 8.0), 1M | 100Mm | 10ml |
| 2 | EDTA (Ph 8.0), 0.5 M | 20mM | 4ml |
| 3 | B- Mercaptoethanol | 0.2% | 200µl |
| 4 | NaCl, 4M | 1.4 M | 35ml |
| 5 | CTAB | 1% | 1gm |

*B-Mercaptoethanol was added just prior to placement of DNA extraction buffer in water bath for incubation*

**Table 2. List of SSR markers used for hybrid testing**

| SSR Marker | Chromosome No. | Forward/ Reverse | Sequence | Annealing temp.(°C) |
|---|---|---|---|---|
| *Xgwm273* | 6D | F | AATTCAGAGAAACACACCTCCCTTTTA | 63.3 |
| | | R | ACTCCATCAACCCCGTTCATTA | |
| *Xgwm582* | 1B | F | AAGCACTACGAAAATATGAC | 53.5 |
| | | R | TCTTAAGGGGTGTTATCATA | |
| *Xbarc188* | 1B | F | CGTGAGATCATGTTATCAGGACAAG | 67.5 |
| | | R | GCGTTGAAAGGTGTTAGTGGGATGG | |
| *Xgwm11* | 1B | F | GGATAGTCAGACAATTCTTGTG | 60.2 |
| | | R | GTGAATTGTGTCTTGTATGCTTCC | |
| *Xcfd84* | 4D | F | GTTGCCTCGGTGTCGTTTAT | 63.5 |
| | | R | TCCTCGAGGTCCAAAACATC | |
| *Xbarc61* | 1B | F | TGCATACATTGATTCATAACTCTCT | 60.8 |
| | | R | TCTTCGAGCGTTATGATTGAT | |
| *Xbarc268* | 3B | F | GCG ATTCCTTTGTTCCTTCCCCATAC | 72.6 |
| | | R | GCAGCATGTCTAGCCAACTTGTCGTG | |
| *Xwmc617* | 4B | F | CCACTAGGAAGAAGGGGAAACT | 63.4 |
| | | R | ATCTGGATTACTGGCCAACTGT | |
| *Xgwm456* | 3D | F | TCTGAACATTACACAACCCTGA | 62.8 |
| | | R | TGCTCTCTCTGAACCTGAAGC | |
| *Xbarc330* | 5A | F | GCACTAAGCGCTCTTTATTTAC | 60.0 |
| | | R | CCTGCATCTGGTATGGAGA | |
| *Xgwm274* | 7B | F | AACTTGCAAAACTGTTCTGA | 58.6 |
| | | R | TATTTGAAGCGGTTTGATTT | |
| *Xbarc207* | 5B | F | CCTTCACAGCCCAACAATCAACAAAC | 77.0 |
| | | R | CGTCTAGGGTTCATCCGCCACTG | |
| *Xgwm18* | 1B | F | TGGCGCCATGATTGCATTATCTTC | 62.4 |
| | | R | GGTTGCTGAAGAACCTTATTTAGG | |
| *Xwmc331* | 4D | F | CCTGTTGCATACTTGACCTTTTT | 62.9 |
| | | R | GGAGTTCAATCTTTCATCACCAT | |
| *Xgwm130* | 7A | F | AGCTCTGCTTCACGAGGAAG | 64.2 |
| | | R | CTCCTCTTTATATCGCGTCCC | |
| *Xgwm131* | 7B | F | AATCCCCACCGATTCTTCTC | 64.0 |
| | | R | AGTTCGTGGGTCTCTGATGG | |
| *Xwmc89* | 4A | F | ATGTCCACGTGCTAGGGAGGTA | 66.2 |
| | | R | TTGCCTCCCAAGACGAAATAAC | |
| *Xgwm153* | 1B | F | GATCTCGTCACCCGGAATTC | 63.3 |
| | | R | TGGTAGAGAAGGACGGAGAG | |
| *Xwmc48* | 4B | F | GAGGGTTCTGAAATGTTTTGCC | 65.4 |
| | | R | ACGTGCTAGGGAGGTATCTTGC | |
| Xbarc137 | 1B | F | GGCCCATTTCCCACTTTCCA | 52.0 |
| | | R | CCAGCCCCTCTACACATTTT | |

## 2.5 PCR Conditions for SSR Analysis

Polymerase chain reaction (PCR) was performed in a reaction mixture containing 15 ng of template DNA, 100 µM of each dNTP, 10x *Taq* buffer, 1.5 mM MgCl2, 0.5U of *Taq* polymerase and 10 µl SSR primer in a thermal cycler (Thermo Hybaid®). Amplification reactions were initiated by a 5 minute pre-denaturation step at 95°C followed by 40 cycles of DNA denaturation at 94°C for 1 minute, primer annealing at 58°C for 30 seconds and DNA extension at 72°C for 1 minute. A final extension step at 72°C for 5 minutes was performed after 40 cycles. PCR amplified products were checked on 2% agarose gels at 50V in 0.5x TBE buffer. After electrophoresis, gels were scanned for documentation. The sizes of the amplified fragments were estimated with the help of the software utility of the gel documentation system using 20-bp DNA ladder (MBI Fermentas, Maryland, USA) as the standard size.

## 3. RESULTS AND DISCUSSION

Seed purity testing is required in seed production, market supervision and breeding research programs. SSR marker-based purity testing may have great advantage in saving time with high degree of certainty [13]. In this investigation, among the 20SSR markers tested, only 8 markers viz., Xwmc617, Xwmc457, Xwmc48, Xgwm153, Xbarc61, Xgwm273, Xbarc268 and Xgwm274 loci were able to generate complementary banding pattern between hybrids and its corresponding parental lines table (Fig. 1, Table 3& Table 4). The loci Xbarc61amplified a specific allele size of 220bp in seed parent (GW 273) and $F_1$ hybrids but not in their pollen parent. Further, Xbarc61 also amplified allele size of 260 bp in pollen parent JW 3336. The same allele size of 260bp was also expressed in F1 hybrid, but not in the female parent (GW 273). These two bands of allele size 220bp and 260bp were also found in hybrid 1(GW 273 X JW 3336), thus confirming that these hybrid was produced from the cross combination of GW 273 X JW 3336. Similarly,Xbarc61 also amplified allele size of 260 bp in pollen parent, 220bp in seed parent and two bands of allele size 220bp and 260bp in their hybrids H2, H3, H9, H10, H11, H24, H37, H38, H39 and H40.Thus, confirming that these hybrids were produced from the cross combination of GW 273 X JW 3288, GW 273X MP 3269, GW 366 X JW 3336, GW 366 X JW 3288, GW 366 X MP 3269, JW 1203 X JW 3211, HI 1544 X JW 3336, HI 1544 X JW 3288 and HI 1544 X MP 3269respectively (Fig. 1). Similarly, Xgwm273 loci amplified allele size of 120 bp in female parent (GW 273), F1 hybrid and it was absent in pollen parent. On the other hand, the pollen parent (JW 3211) generated an amplicon at 160 bp, which was absent in the female parent. However, F1 hybrid showed the allele of both parents confirming the heterozygosity by generating two bands at 120 and 160 bp. Xgwm273 also amplified allele size of 120 and 160 bp in hybrids H4, H5, H6, H7, H8, H12, H16, H17, H18, H19 and H20, thus, confirming that these hybrid was produced from the cross combination of GW 273 X JW 3211, GW 322 X JW 3336, GW 322X JW 3288, GW 322 X MP3269, GW 322 X JW 3211, GW 366 X JW 3211, JW 1201 X JW 3211, JW 1202 X JW 3336, JW 1202 X JW 3288, JW 1202 X MP 3269 and JW 1202 X JW 3211, respectively.

The loci Xwmc48 amplified a specific allele size of 125bp in seed parent (JW 1201, JW 1203) and F1 hybrid but not in their pollen parent. Further, Xwmc48 also amplified allele size of 155 bp in pollen parent JW 3336, JW 3288 and MP 3269. The same allele size of 155bp was also expressed in F1 hybrid, but not in the female parent. These two bands of allele size 125bp and 155bp were found in hybrids H13, H14, H15, H21 H22 and H23, thus, confirming that these hybrids were produced from the cross combination of JW 1201 X JW 3336, JW 1201 X JW 3288, JW 1201 X MP 3269, JW 1203 X JW 3288 and JW 1203 X MP 3269, respectively.

Similarly, Xgwm153loci amplified allele size of 210 bp in female parent (LOK 1), F1 hybrid and it was absent in pollen parent. On the other hand, the pollen parent (JW 3336, JW 3288, MP 3269, JW 3211) generated an amplicon at 240 bp, which was absent in the female parent. However, F1 hybrids (LOK 1 X JW 3336, LOK 1 X JW 3288, LOK 1 X MP 3269 and LOK 1 X JW 3211) showed the allele of both parents confirming the heterozygosity by generating two bands at 210 and 240 bp.

The loci Xgwm274 amplified a specific allele size of 250 bp in seed parent (HD 2864) and F1 hybrid but not in their pollen parent. Further, Xgwm274 also amplified allele size of 300 bp in pollen parent (JW 3336, JW 3288, MP 3269). The same allele size of 300 bp was also expressed in F1 hybrid, but not in the female parent. These two bands of allele size 250bp and

300bp were found in hybrids H29 and H30, thus confirming that these hybrids were produced from the cross combination of HD 2864 X JW 3336 and HD 2864 X JW 3288.

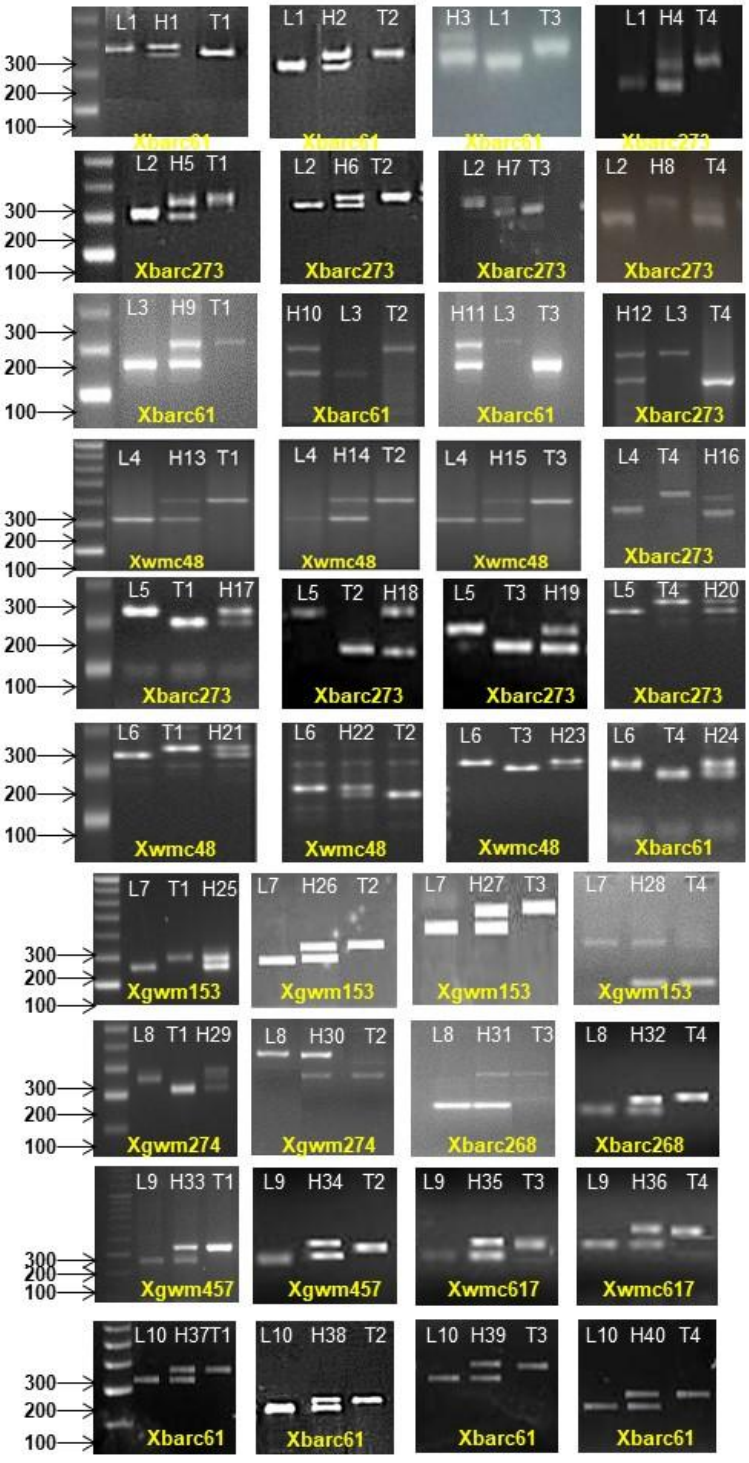


**Fig. 1. Polymorphic SSR markers profiles confirming hybridity in wheat. M= 100bp ladder, L1 -L10= Lines, T1- T4= Tester, H1- H40= Hybrids**

**Table 3. List of SSR markers (used in authentication of hybrids) and linked genes associated to traits**

| Marker | CH. No. | Forward/ Reverse | AntiSequence (5'→3') | Linked to trait and gene | Previous studies | Band size |
|---|---|---|---|---|---|---|
| Xgwm273 | 7B | F | ATTGGACGGACAGATGCTTT | Black stem rust (*Sr17*) | Arora et al., 2014 | 170 |
| | | R | AGCAGTGAGGAAGGGGATC | | | 200 |
| Xwmc457 | 4D | F | CTTCATGAATCAAAGCAAGCAC | Male sterility gene (ms2) | Somers and Isaac | 159 |
| | | R | CATCCATGGCAGAAACAATAGC | | (2004) | 170 |
| *Xgwm*153 | 1B | F | GATCTCGTCACCCGGAATTC | Fertility restoration (*Rf1*) | Arora et al., 2014 | 170 |
| | | R | TGGTAGAGAAGGACGGAGAG | | | 220 |
| Xbarc61 | 1B | F | TGCATACATTGATTCATAACTCTCT | Fertility restoration (*Rf3*) | Prakash et al., 2012 | 218 |
| | | R | TCTTCGAGCGTTATGATTGAT | | | 221 |
| Xbarc268 | 1B | F | GCGATTCCTTTGTTCCTTCCCCATAC | Restorers for *T. timopheevic*ytoplasm | Zhou et al., 2005 | 203 |
| | | R | GCAGCATGTCTAGCCAACTTGTCGG | (*Rf3*) | | 225 |
| Xwmc617 | 4D | F | CCACTAGGAAGAAGGGGAAACT | associated with | Prakash et al., 2012 | 215 |
| | | R | ATCTGGATTACTGGCCAACTGT | Lipoxygenaseactivity(*Lpx*) | | 218 |
| Xwmc48 | 4D | F | GAGGGTTCTGAAATGTTTTGCC | Male sterility ms2 | Somers and Isaac | 100 |
| | | R | ACGTGCTAGGGAGGTATCTTGC | | (2004) | 190 |
| Xgwm274 | 3B | F | AACTTGCAAAACTGTTCTGA | Resistance to *Fusariumgraminearum* | Roder et al. (1998) | 200 |
| | | R | TATTTGAAGCGGTTTGATTT | (*Fhs.ndsu*) | | 280 |

**Table 4. Polymorphic SSR markers with different band size between parental lines for hybrid testing**

| Hybrid No | Hybrid | Parent line code | SSR Marker | Band size |
|---|---|---|---|---|
| H1 | GW-273 X JW-3336 | L1 | Xbarc61 | 220 |
| | | T1 | | 260 |
| H2 | GW-273 X JW-3288 | L1 | Xbarc61 | 220 |
| | | T2 | | 260 |
| H3 | GW-273 X MP-3269 | L1 | Xbarc61 | 220 |
| | | T3 | | 260 |
| H4 | GW-273 X JW-3211 | L1 | Xgwm273 | 120 |
| | | T4 | | 160 |
| H5 | GW-322 X JW-3336 | L2 | Xgwm273 | 120 |
| | | T1 | | 160 |
| H6 | GW-322 X JW-3288 | L2 | Xgwm273 | 120 |
| | | T2 | | 160 |
| H7 | GW-322 X MP-3269 | L2 | Xgwm273 | 120 |
| | | T3 | | 160 |
| H8 | GW-322 X JW-3211 | L2 | Xgwm273 | 120 |
| | | T4 | | 160 |
| H9 | GW-366 X JW-3336 | L3 | Xbarc61 | 220 |
| | | T1 | | 260 |
| H10 | GW-366 X JW-3288 | L3 | Xbarc61 | 220 |
| | | T2 | | 260 |
| H11 | GW-366 X MP-3269 | L3 | Xbarc61 | 220 |
| | | T3 | | 260 |
| H12 | GW-366 X JW-3211 | L3 | Xgwm273 | 120 |
| | | T4 | | 160 |
| H13 | JW-1201 X JW-3336 | L4 | Xwmc48 | 125 |
| | | T1 | | 155 |
| H14 | JW-1201 X JW-3288 | L4 | Xwmc48 | 125 |
| | | T2 | | 155 |
| H15 | JW-1201 X MP-3269 | L4 | Xwmc48 | 125 |
| | | T3 | | 155 |
| H16 | JW-1201 X JW-3211 | L4 | Xgwm273 | 120 |
| | | T4 | | 160 |
| H17 | JW-1202 X JW-3336 | L5 | Xgwm273 | 120 |
| | | T1 | | 160 |
| H18 | JW-1202 X JW-3288 | L5 | Xgwm273 | 120 |
| | | T2 | | 160 |
| H19 | JW-1202 X MP-3269 | L5 | Xgwm273 | 120 |
| | | T3 | | 160 |
| H20 | JW-1202 X JW-3211 | L5 | Xgwm273 | 120 |
| | | T4 | | 160 |
| H21 | JW-1203 X JW-3336 | L6 | Xwmc48 | 125 |
| | | T1 | | 155 |
| H22 | JW-1203 X JW-3288 | L6 | Xwmc48 | 125 |
| | | T2 | | 155 |
| H23 | JW-1203 X MP-3269 | L6 | Xwmc48 | 125 |
| | | T3 | | 155 |
| H24 | JW-1203 X JW-3211 | L6 | Xbarc61 | 220 |
| | | T4 | | 260 |
| H25 | LOK-1 X JW-3336 | L7 | Xgwm153 | 210 |
| | | T1 | | 240 |
| H26 | LOK-1 X JW-3288 | L7 | Xgwm153 | 210 |
| | | T2 | | 240 |

| Hybrid No | Hybrid | Parent line code | SSR Marker | Band size |
|---|---|---|---|---|
| H27 | LOK-1 X MP-3269 | L7 | Xgwm153 | 210 |
| | | T3 | | 240 |
| H28 | LOK-1 X JW-3211 | L7 | Xgwm153 | 210 |
| | | T4 | | 240 |
| H29 | HD-2864 X JW-3336 | L8 | Xgwm274 | 250 |
| | | T1 | | 300 |
| H30 | HD-2864 X JW-3288 | L8 | Xgwm274 | 250 |
| | | T2 | | 300 |
| H31 | HD-2864 X MP-3269 | L8 | Xbarc268 | 250 |
| | | T3 | | 300 |
| H32 | HD-2864 X JW-3211 | L8 | Xbarc268 | 250 |
| | | T4 | | 300 |
| H33 | HD-2932 X JW-3336 | L9 | Xwmc457 | 130 |
| | | T1 | | 180 |
| H34 | HD-2932 X JW-3288 | L8 | Xwmc457 | 130 |
| | | T2 | | 180 |
| H35 | HD-2932 X MP-3269 | L8 | Xwmc617 | 130 |
| | | T3 | | 180 |
| H36 | HD-2932 X JW-3211 | L8 | Xwmc617 | 130 |
| | | T4 | | 180 |
| H37 | HI-1544 X JW-3336 | L10 | Xbarc61 | 220 |
| | | T1 | | 260 |
| H38 | HI-1544 X JW-3288 | L10 | Xbarc61 | 220 |
| | | T2 | | 260 |
| H39 | HI-1544 X MP-3269 | L10 | Xbarc61 | 220 |
| | | T3 | | 260 |
| H40 | HI-1544 X JW-3211 | L10 | Xbarc61 | 220 |
| | | T4 | | 260 |

*L series – female lines, T series – male lines and H series - hybrids*

Xbarc268 loci amplified allele size of 250 bp in female parent (HD 2864), F1 hybrid and it was absent in pollen parent. On the other hand, the pollen parent (MP 3269, JW 3211) generated an amplicon at 300 bp, which was absent in the female parent. However, F1 hybrids of cross combination HD 2864 X MP 3269 and HD 2864 X JW 3211 showed the allele of both parents confirming the heterozygosity by generating two bands at 250 and 300 bp.

The loci Xwmc457 amplified a specific allele size of 130 bp in seed parent (HD 2932) and F1 hybrid but not in their pollen parent. Further, Xwmc457 also amplified allele size of 180 bp in pollen parent JW 3336 and JW 3288.The same allele size of 180 bp was also expressed in F1 hybrid, but not in the female parent. These two bands of allele size 130 bp and 180 bp were found in hybrids H33 and H34, thus confirming that these hybrids were produced from the cross combination of HD 2932 X JW 3336 and HD 2932 X JW 3288, respectively.Xwmc617 loci amplified allele size of 130 bp in female parent (HD 2932), F1 hybrid and it was absent in pollen parent. On the other hand, the pollen parent (MP 3269, JW 3211) generated an amplicon at 180 bp, which was absent in the female parent. However, F1 hybrids (H35, H36) showed the allele of both parents confirming the heterozygosity by generating two bands at 130 and 180 bp.

All the forty hybrids showed similar banding pattern as parental lines, this proved the purity of hybrids in all these cross combination and also provides important information about heterozygosity of hybrids. This finding is also in agreement with the statement of Morgante and Olivieri [14] that SSR markers are very useful due to their co-dominant and multi allelic nature, higher reproducibility, relative abundance and good genome coverage. Similarly, Ahmed et al. [15] used SSR primer Xgwm-314 to find out the co-dominant loci in the hybrids (LU26S x 9272 and Mehrajx 9381) and single dominant loci in parents. It is suggested that a single codominant marker is sufficient to distinguish false hybrids from real hybrids [16,17]. SSR markers can be used in program of hybrid wheat management especially to detect/monitor contamination by using homozygosity of parent lines producing

hybrids. Since molecular markers are very sensitive and specific thus even lowest contamination can be detected which is not feasible otherwise by phenotypic methods. In such program, SSRs have also been used to detect cytoplasmic sterility genes/restorer genes in hybrid wheat [18]. Molecular markers can give best empirical evaluation of genetic relatedness rather than DUS phenotypic features [19].

## 4. CONCLUSION

All the forty hybrids showed similar banding pattern as parental lines, this proved the purity of hybrids in all this cross combination and also provides important information about heterozygosity of hybrids besides seed purity testing of hybrid without anyambiguity. This research showed that SSR markers are fast and effective having high accuracy in results and reliable assessment of hybrid purity of wheat. Effective markers identified in this study would be a good option for establishment of a seed quality control system to be applied for seed and hybrid purity testing in commercial seed production of wheat.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## ACKNOWLEDGEMENTS

The author would like to acknowledge the Head of Department, Plant Breeding and Genetics, JNKVV, Jabalpur for his valuable support and facilitation.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Consortium IWGS. A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. Science. 2014;345:1251788. Available:10.1126/science.1251788
2. Gowda M, Longin CFH, Lein V,Reif JC. Relevance of specific versus general combining ability in winter wheat. Crop Science. 2012;52:2494–2500.
3. Wang LX, Li HB, Liu LH, Pang BS, Zhao CP. Assessment of wheat variety stability based on SSR markers, Euphytica. 2014a; 195:435-452. Available:http://dx.doi.org/10.1007/s10681-013-1006-z
4. Fujita Y, Fukuoka H, Yano H. Identification of wheat cultivars using EST–SSR markers. Breed. Sci. 2009;59:159–167. Available:10.1270/jsbbs.59.159
5. Garg A, Singh AK, Prabhu KV,Mophapatra T, Tyagi NK,Nandkumar N, Singh R, Zaman FU. Utility of fertility restorer gene linked marker for testing genetic purity of hybrid seeds in rice (*Oryza sativa* L.). Seed science and technology. 2006;34:9-18.
6. Malik R, Tiwari R, Arora A, Kumar P, Sheoran S, Sharma P, et al. Genotypic characterization of elite Indian wheat genotypes using molecular markers and their pedigree analysis. Aust. J. Crop Sci. 2013;7:561–567.
7. Wang XL, Jun QUI, Chang LF, Zhao CP. Assessment of wheat variety uniformity using SSR markers. Molecular Plant Breeding; 2015. DOI: 10.5376/mpb.2015.06.0021
8. Roder MS, Korzun V, Wendehake K, Piaschke J, TixierMH, Leory P,Ganal MW. A micro satellite map of wheat. Genetics. 1998;149:2007-2023.
9. Singh K, Khara D, Rao S. Identification of lentil varieties by seed protein electrophoresis. Seed Research. 2006; 34(1):40-44.
10. Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW. Ribosomal DNAsepacer-length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci. 1984;81: 8014–8019.
11. Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB. Development and mapping of microsatellite (SSR) markersin wheat. Theoretical and Applied Genetics. 2005; 110(3):550-60.

12. Gupta PK, Balyan HS, Edwards KJ, Isaac P, Korzun V, Röder M, Gautier M F, Joudrier P, Schlatter AR, Dubcovsky J, De la Pena RC, Khairallah M, Penner G, Hayden MJ, Sharp P, Keller B, Wang RCC, Hardouin JP, Jack P, Leroy P. Genetic mapping of 66 new microsatellite (SSR) in bread wheat. Theoretical and Applied Genetics. 2002;105:413-422.
13. Wang LX, Li HB, Gu TC, Liu LH, Pang BS, Qiu J, et al. Assessment of wheat variety stability using SSR markers. Euphytica. 2014b;195:435–452.
DOI: 10.1007/s10681-013-1006-z
14. Morgante M, Olivieri AM. PCR-amplified microsatellites as markers in plant genetics. Plant J.1993;3(1):175–182.
15. Ahmed MS, Khaliq I, Farooq J, Awan SI, Ahmed N and Awan FS. Assessment of the combining ability and authentication of $F_1$hybrids using SSR markers in wheat (*Triticum aestivum* L.).Front. Agric. China. 2011;5(2):135–140.
16. Nandakumar N, Singh AK, Sharma RK, Mohapatra T, Prabhu KV, Zaman FU. Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. Euphytica. 1993;136:257–264.
17. Yashitola J, Thirumurugan T, Sundaram RM, Naseerullah MK, Ramesha MS, Sarma NP, Stone RV. Assessment of purity of rice hybrids using microsatellite and STS markers. Crop Science. 2002;42: 1369–1373.
18. Sinha P, Tomar S, Singh VK, Balyan H. Genetic analysis and molecular mapping of a new fertility restorer gene Rf8 for *Triticum timopheevi* cytoplasm in wheat (*Triticum aestivum* L.) using SSR markers. Genetica. 2013;141:431–441.
Available:10.1007/s10709-013-9742-5
19. Heckenberger M, Bohn M, Melchinger A. Identification of essentially derived varieties obtained from biparental crosses of homozygous lines. Crop Sci. 2005;45, 1120–1131.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://www.sdiarticle4.com/review-history/62758*