Detection of True Hybrids in Pearl Millet Cross CombinationsbyEmploying SSR Molecular Markers

ABSTRACT

Pearl millet (*Pennisetum glaucum* L. R. Br.), a crucial staple food and significant cereal crop, is gaining prominence due to its versatile applications as feed, food, and fodder. Heterosis of this crop has been extensively harnessed to increase productivity. Hybrid variants exhibit superior grain and stover yields compared to open-pollinated varieties.The primary aim of this investigation was to evaluate and confirm authentic hybrids within three resultant F_1 progenies. The assessment of parents and F_1 hybrids was carried out during the *Kharif* 2021for the purpose of accurate discrimination and rapid verification of true hybrids by employing 20 SSR molecular markers.The experimental materials consisted of three distinct cytoplasmic male sterile (CMS) linesincluding ICMA 843-22, ICMA 04999, and ICMA 02333, used as female parents along with three fertility restorers, *viz*., ICMR 01004, ICMR 20233, and ICMR 20342, were utilized as male parents. The analysis of SSR profiles was based on distinctive banding patterns, resulting in unique profiles for the hybrids. The amplified fragment sizes ranged between 90 to 300 base pairs (bp), effectively enabling the differentiation of authentic hybrids. Within the specific crosses, the percentage of polymorphism was determined 75% for the cross ICMA 843-22 × ICMR 01004, 80% for the cross combination ICMA 04999 × ICMR 20233, and 75% for the cross ICMA 02333 × ICMR 20342. The true hybrids were calculated using hybrid purity percentage formula using heterozygous banding patten among total plants evaluated. Among a total of 100 F_1 plants, 85, 86, and 88 plants were accurately identified as true hybrids in the respective crosses *i.e*., ICMA 843-22 × ICMR 01004, ICMA 04999 × ICMR 20233, and ICMA 02333 × ICMR 20342. The identified markers hold significant potential for applications such as hybridity test, genetic purity assessments, diverse germplasm identification, and DNA fingerprinting endeavors in future.

Key words: *Pennisetum glaucum*, SSR (Simple sequence repeat), Hybrids, Polymorphism

INTRODUCTION

Pearl millet (*Pennisetum glaucum*(L.) R. Br.) holds a significant position as a cereal crop, extensively cultivated in Asia and Africa, contributing substantially to global millet production [1]. In these regions, it constitutes approximately half of the millet output worldwide [2]. Among food crops in India, pearl millet stands as the fourth most cultivated after rice, wheat, and maize [3]. Its versatility as both cattle feed and fodder further augments its value [4]. The nutritional profile of pearl millet underscores its importance [5]. In the Indian context, it holds a crucial role as a short-duration food and fodder crop [6]. It encompasses noteworthy attributes such as elevated nutritional content, including energy, carbohydrates, modest fat content (5-7%), dietary fiber (1.2g/100g), α-amylase activity, and quality protein (9-13%) [7]. Additionally, it boasts antioxidants like ferulic acid and coumaric acids, vitamins, and essential micronutrients such as iron, zinc, calcium, magnesium, copper, manganese, and phosphorus [8]. This rich composition substantiates its role in bolstering food and nutritional security, earning it the moniker "nutri-cereal"[9]. Pearl millet thrives in regions characterized by elevated temperatures, diminished soil fertility, and drought conditions [10,2,5]. It excels even in scenarios of low soil pH or pronounced salinity [2,5]. Its growth capabilities extend to environments where other cereal crops like maize or wheat might struggle due to its remarkable resilience to challenging growing conditions [11].

Insufficient genetic variability within cultivated millet species and a dearth of polymorphic markers contribute to the absence of diagnostic morphological indicators for confirming hybridity in pearl millet [12-14,7]. This constitutes a prominent challenge in validating hybrid status [7]. The consequences of this limitation include the potential misclassification of false hybrids as authentic ones, leading to discrepancies in breeding programmes and the wastage of valuable resources and time [15]. While traditional breeding techniques have demonstrated success in developing resistant pearl millet varieties, they are laborious, time-intensive, resource-demanding, and susceptible to environmental influences. Thus, the creation of resilient varieties necessitates an approach that offers rapid development with minimal environmental impact [10]. The introduction of disease resistance into existing favored cultivars via genetic methods has proven efficacious [12-14]. This has led to advancements in enhancing various biotic and abiotic traits in pearl millet, particularly through the utilization of the cytoplasmic male sterility system in conjunction with marker-assisted

selection breeding strategies [7]. Molecular markers associated with specific traits have gained prominence and are increasingly applied in diverse crop species [16-37]. Capitalizing the heterosis of pearl millet has been a pivotal avenue to booster productivity. An important stride in pearl millet hybridization involves the identification of the A_1 cytoplasmic nuclear male sterility system, culminating in the development of commercially viable male sterile lines (A-lines) [7].

Molecular markers constitute pivotal tools for the breeding selection of genotype discrimination, and the exploration of plant genome organization and evolution[16-37]. Among these markers, Simple Sequence Repeats (SSRs) offer distinct advantages, being abundant, reproducible, co-dominant, and widely dispersed across crop genomes. Moreover, their application demands a minute quantity of DNA for polymerase chain reaction (PCR) analysis, facilitating the detection of polymorphisms [38, 16-37]. SSRs emerge as invaluable resources for evaluating genetic diversity, identifying potential allelic sources for the augmentation of key traits, and delving into the genomic evolution and historical trajectory of cultivars [39-40].Numerous studies have spotlighted the utility of DNA markers in millet cultivar identification, exemplified by Avadh *et al*. [41], Kumar *et al*. [42] , and Nagawade *et al*. [43]. Molecular markers have been harnessed across diverse crops to establish DNA fingerprints [44], facilitate seed purity assessments, underpin variety registration systems, support Distinctness, Uniformity, and Stability (DUS) examinations [40], aid in variety protection [39], and enable hybridity testing [45].The present investigation conductedwith aim to assess the purity of true hybrids, and their parental lines using SSR molecular markers.

MATERIALS AND METHOD

The experimental materials wereobtained from ICRISAT, Hyderabad, Indiaconsisted of three different cytoplasmic male sterile (CMS)lines *i.e*.,ICMA 843-22, ICMA 04999 and ICMA 02333 containing A_1 and A_4 cytoplasm and used as female parents along with three fertility restorers *viz*.,ICMR 01004, ICMR 20233 and ICMR 20342were used as male. Hybridization was employed to generate $3F_1$s namely: ICMA 843-22× ICMR 01004, ICMA 04999 × ICMR 20233 and ICMA 02333 × ICMR 20342.The parents were raised at ICRISAT, Hyderabad during *kharif* 2020.Six parents and three F_1S were subjected to field trials in *Kharif*2021 at Agriculture Farm, Department of Genetics and Plant Breeding, College of Agriculture, Rajmata Vijyaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh, India. The parents and F_1populations were

raised on a four-meter-long plot in three rows containing approximately 30–40 plants each. Molecular analysis work was conducted at Plant Molecular Biology Laboratory, Department of Plant Molecular Biology & Biotechnology, College of Agriculture, Rajmata Vijyaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh, India.

Hybridization Process

These male and female parents constituted crossing blocksand each female was crossed withthe males and generated three different cross combinations *viz.*,ICMA843-22 × ICMR01004, ICMA04999 × ICMR20233 and ICMA02333 × ICMR 20342.Individual plants were used for making plant × plant crosses to produce these F_1s. Both male and female parents were bagged before stigma emergence. At the time of crossing, bagged panicles of female parents were observed for complete stigma emergence. Similarly bagged panicles of male parent were observed for pollen load and pollens were collected from the desired parent between 10.00 to 11.30 AM. The pollens were dusted on the panicle of the female parent in which stigma completely emerged and pollinated panicle was again covered. After forty days of pollination, crossed panicles were harvested and dried. Then panicles were threshed to get F_1 seeds.

Genomic DNA Isolation and Quantification

Genomic DNA was extracted from young and healthy leaves of 12-15 days-old seedling from the selected parents and their respective F_1 offspring, employing the Cetyl trimethyl ammonium bromide (CTAB) method with minor modifications as proposed by Ambawat *et al*. [46]. The extracted DNA was subsequently quantified through electrophoresis on a 0.8% agarose gel. Following quantification, the obtained DNA was diluted to achieve a final concentration of 10 ng/µl, rendering it suitable for subsequent polymerase chain reaction (PCR) assays. The resultant DNA samples were then stored at -20°C to ensure their preservation for future utilization.

DNA Profile Employing SSRs

The polymerase chain reaction (PCR) procedure was executed using a 96-well thermal cycler (Agilent Technologies) with a panel of 20 previously established SSR primers, as documented in the reported literature (Table1). The PCR master mix, constituting a 10 µl reaction volume, comprised the following components: 10 mM Tris HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 200 mM each of dNTPs, 0.4 µM 10-mer primer, 1 unit of Taq DNA polymerase, and 10 ng of

DNA template. The amplification process began with an initial denaturation phase at 94°C for 5 minutes, succeeded by 35 cycles. Each cycle consisted of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and primer extension at 72°C for 1 minute, as previously outlined by Ambawat *et al*. [47]. A final extension step was conducted for 10 minutes at 72°C, succeeded by a holding period at 4°C. Subsequently, the amplified products were subjected to analysis on a 3.5% agarose gel, with the resulting DNA bands visualized through a Gel Doc system and subsequently recorded. The final scoring was predicated on the discernible presence or absence of specific alleles.

Data Analysis

Analysis of genetic data was performed using Microsoft excel sheet. Scoring was done based on the banding pattern on the agarose gel electrophoresis. Double bands (Heterozygous condition) are considered as true hybrids whereas single (homozygous condition) consider as false indicator.

The hybrid purity percentage is calculated based on the banding pattern observed in individual plant samples, using the following formula:

Hybrid Purity (%) = (Number of True Hybrid Plants/Total Number of Plants)×100

In this formula:

Number of true hybrid plants refers to the count of plants that exhibit the heterozygous banding pattern and total numbers of plants represents the total count of all plants in the sample.

RESULTS AND DISCUSSION

Confirmation of True F_1Hybrids

The identification of true hybrids holds significance in the realm of pearl millet due to its cross-pollinating nature, characterized by a relatively low rate of selfing, ranging from 5% to 10%. This breeding context frequently leads to the occurrence of extensive true hybrids, albeit a minority that has the potential for self-pollination. To achieve accurate differentiation between heterozygotes and homozygotes, SSR markers were employed for authentic hybrid identification [7]. The selection encompassed a total of 20 SSR primers, which were employed to assess the degree of polymorphism among six parental combinations.In the experimental progression,

approximate 100 hybrid seeds from each cross were selected, and these were sown in the field. The assessment of hybridity was executed through the application of SSR primers. In the identification process, individuals were validated based on the display of heterozygosity at specific loci, signifying the presence of both parents'specific alleles. In contrast, individuals manifesting the presence of only one parental allele were ascertained as off-types, signifying divergence from the true hybrid state [48].

Cross ICMA 843-22× ICMR 01004

The successful isolation of high-quality genomic DNA, characterized by distinct and well-defined bands, was achieved from the fresh and young leaves of two parent genotypes, ICMA 843-22 and ICMR 01004, alongside their respective 100 F_1 progenies. Employing a set of 20 SSR primers for PCR amplification facilitated the parental polymorphism in the pearl millet. Out of 20 SSR markers only 15 SSR markers showed polymorphism for respective parents. The amplification of all 15 primers substantiated the existence of a substantial genetic polymorphism, granting rise to unique banding patterns. The SSR markers were analyzed based on pattern of bands and they showed unique amplicons between 90 to 300 bp. Analysis of F_1using 15 primers displayed a unique profile. The analysis of SSR profiles, contingent upon banding patterns, elucidated amplicons ranging between 90 to 300 base pairs. Specifically, the assessment of F_1 individuals utilizing 15 primers generated distinct profiles, as exemplified in Table 2 and Fig.1. Among the 20 primers screened, 15 SSR primers (75%) including ICMP3088, IPES0019, CTM27, IPES0163, ICMP3066, CTM1, CTM10, CTM57, CUMP016, ICMP3086, ICMP3088, ICMP3092, ICMP4006, ICMP4010, and ICMP4012 exhibited polymorphism between the parental genotypes. These polymorphic primers were subsequently employed for the hybridity test. The detection of heterozygosity at specific loci, encompassing two distinct alleles from each parent, validated the true hybrid status. Conversely, individuals exclusively displaying one parental allele were ascribed as off-types. Out of 100 F_1 plants assessed, 85 were identified as true hybrids, characterized by the presence of heterozygous bands.

This conspicuous polymorphic variation serves as an indicator of the considerable genetic diversity encompassed by the studied hybrids. This was obvious as different parental lines have been used in the development of this hybrid. Being allogamous in nature, pearl millet genotypes are highly heterogeneous reflecting high variability within and among the genotypes. Factors

such as protogyny and the temporal interval between stigma emergence and anther dehiscence contribute to favorable conditions for complete cross-pollination, thus accentuating genetic diversity. Similar observations of highest polymorphism havealso been documented by Kaushik*et al*. [49] in pearl millet utilizing SSR markers.

Cross ICMA 04999 × ICMR 20233

Similarly, analysisof F_1 using the same 20 primers displayed a unique profile for F_1 plants and parents (Table 3;Fig. 2). Out of the 20 primers used, 16 SSR primers (80%) *viz*.,ICMP3088, IPES0019, CTM27, IPES0163, ICMP3066, CTM1, CTM10,CTM57,CUMP016, ICMP3086, ICMP3088, ICMP3092, ICMP4006, ICMP4010, ICMP3094 and ICMP4012 were found polymorphic between the parentsICMA 04999 and ICMR 20233, the amplicon size varied from 90 to 300 bp. 16 SSR primers were used for hybridity confirmation on 100 F_1s plants 86 plants were confirmed as true hybrids containing heterozygous bands. The heterozygous at the respective loci for two specific alleles of both parents confirmed the true hybrids.

Cross Combination ICMA 02333 × ICMR 20342

Similarly, employing the same set of 20 primers for analysis yielded a distinct profile for parents ICMA 02333 and ICMR 20342 are presented in Table 4 and Fig. 3. Among the set of 20 primers, only 14 SSR primers(70%)*i.e.*,ICMP3088, IPES0019, CTM27, IPES0163, ICMP3066, CTM10, CTM57, ICMP3086, ICMP3088, ICMP3092, ICMP4006, ICMP4010, ICMP3094, and ICMP4012exhibited polymorphism between the parental genotypes ICMA 02333 and ICMR 20342. The amplicon sizes spanned from 90 to 300 base pairs.Demonstrating heterozygosity at specific loci, wherein two distinct alleles from both parent lines were evident, served as confirmation of true hybridity. Conversely, individuals presenting solely one parental allele were verified as off-types. In the endeavor to ascertain hybridity, 14 SSR primers were employed across 100 F_1 plants, with a total of 88 plants validated as authentic hybrids, characterized by the presence of heterozygous bands.

The employed set of SSR markers in this study holds potential for subsequent applications and could offer valuable insights into the capacity of SSRs to generate distinct DNA profiles within pearl millet. Utilizing SSRs for DNA profiling is a strategic choice, particularly due to the suitability of SSRs for hybridity test. This is underpinned by their heightened accuracy

and efficiency in detecting a substantial array of discrete alleles, as documented by Singh*et al*. [50]. The choice of SSRs as a preferred genotyping marker is grounded in their abundance, substantial allelic variation, co-dominant inheritance pattern, and analytical straight forwardness, as highlighted by Miah *et al*. [51]. Their extensive application across these studies substantiates their role in germplasm identification, cultivar fingerprinting, true hybrid authentication, genetic purity evaluation, parental lineage confirmation, and the delineation of heterotic patterns in hybrids. The identification of true hybrids assumes paramount importance in the establishment of authentic breeding populations. These verified hybrids play a pivotal role in the development of true breeding populations or in mapping endeavors [39,7].

Notably, polymorphic SSR markers have proven instrumental in the identification of true hybrids across various crops, including rice [52], sorghum [53-54], sunflower [55], maize [56], cotton [57], groundnut[58-60,39,7]and more recently in millets. These studies collectively underscore the versatile utility of SSR markers in various domains, spanning genetic purity analysis, germplasm identification, genetic diversity assessment, gene mapping, cultivar fingerprinting, and marker-assisted backcross selection.

CONCLUSION

The results of the present investigation revealed that out of 100 F_1 plants tested from each cross 85, 86, and 88 plants respectivelywere accurately identified as true hybrids at the molecular level and can be used for the development of F_2 mapping population for QTL mapping. A hybridity test in pearl millet is a crucial procedure employed to confirm the authenticity of hybrids resulting from controlled crosses between different parental lines. This test ensures that the identified offspring truly exhibit the genetic characteristics inherited from both parents and are not misidentified due to accidental self-pollination or cross-contamination. The hybridity test typically involves the use of molecular markers, such as SSR, which are highly polymorphic and can accurately differentiate between the alleles inherited from each parent. This test is of great importance in maintaining the genetic purity and authenticity of hybrid seeds, which are extensively used in agriculture to capitalize on the phenomenon of hybrid vigor (heterosis) for enhanced productivity. Ensuring the integrity of hybridity through accurate testing helps prevent the dissemination of falsely labeled seeds and supports successful breeding programmes.

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Table1 List of SSRs markers used for detection of parental polymorphism [7]

S. No.	Primer name	Forward primer sequence (5'-3'	Reverse primer sequence (5'-3')	References
1	ICMP3088	TCAGGTGGAGATCGATGTTG	TTACGGGAGGATGAGGATG	[61]
2	ICMP3086	ACCAAACGTCCAAACCAGAG	ATATCTCTTCGCTGCGGTGT	
3	ICMP3088	TCAGGTGGAGATCGATGTTG	TTACGGGAGGATGAGGATG	
4	ICMP3092	GTTGCTGTCATGTCGTCTGG	CATCATGCCTGTGAGCAATG	
5	ICMP3094	GACCTCGACCTCATCTCCAA	CGACAGCGAACTGGGATTAC	
6	ICMP4006	TGAGGACCGAGAAGAAGCAT	CAACACCCAACAGAAACTGAA	
7	ICMP4010	ATCCCCTACAGCATCAGCAC	CGGCGGAGAGATCTTATTCA	
8	ICMP4012	GACGGACAGCGAGGATAGAG	ACTACTTCGGCAGCCTTCAA	
9	ICMP3066	GGCCCCAAGTAACTTCCCTA	TGTCAGACACAGATGCCACA	
10	IPES0017	CCTATGGCGGCAGAGTAGTG	TTCCGGCACAATTACTTTCA	[62]
11	IPES0035	TGTTGGAAACAAAACCCGAT	ATTACCACGTCTACCTGCCG	
12	IPES0163	AAGATCAAGGCCAGCAACTG	GAGAGTGCACCTGTGCAAAA	
13	PSMP2240	AGCCCAAAAGAAGTGGTCTAAC	CAACCACTAAAGTCTTACTGAACC	[63]
14	CTM27	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT	[64]
15	CTM1	TCTGGGGATTGGCTGGAATTACA	AAGTTGGGTAACGCCAGGGTTTTC	
16	CTM10	GAGGCAAAAGTGGAAGACAG	TTGATTCCCGGTTCTATCGA	
17	CTM57	TGGTGGCAATGCAAGCTACAG	AGCGAGACGATCGACAGGG	
18	CTM9	GCCTCCTCTTGATACCATATT	TAGCCTTGGCTGCTATATTC	
19	CUMP008	GTTGACTACCACTATTATGCTCC	GACCAAGAACTTCATACAATTCAG	[65]
20	CUMP016	CATTTCTCTCGCCAGTGCTC	ATCTCCAGAACCGAGCGCA	

Table2Allelic profile of parent ICMA843-22 (A- Line) and ICMR01004 (R- Line) using 20 SSR primers

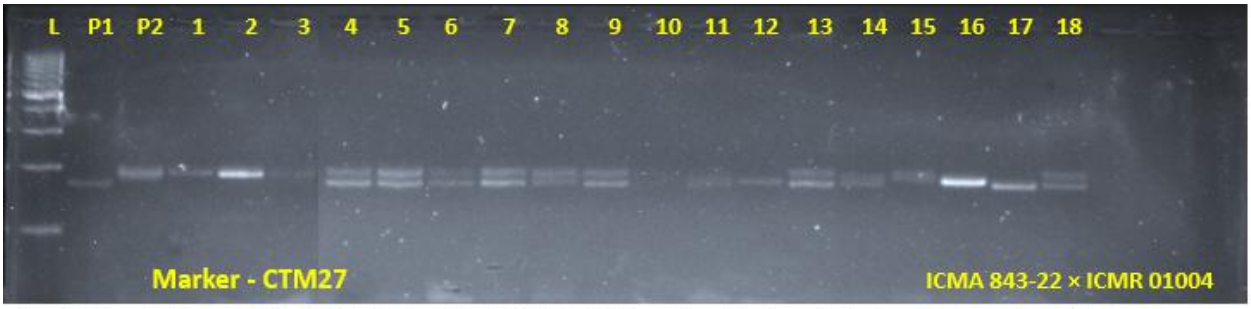
S.No.	Primer Name	Observed band size (bp)			Polymorphism
		(A- Line) ICMA843-22	(R- Line) ICMR01004	F_1-HHB67 IMP-9	
1	ICMP3088	169/169	194/194	149/169	Yes
2	IPES0019	116/116	109/109	116/116	Yes
3	PSMP2240	185/185	185/185	185/185	No
4	CTM27	190/190	200/200	190/200	Yes
5	IPES0163	190/190	180/180	180/190	Yes
6	ICMP3066	210/210	200/200	210/210	Yes
7	CTM1	145/145	150/150	145/150	Yes
8	CTM10	190/190	210/190	210/190	Yes
9	CTM57	200/200	215/215	200/215	Yes
10	CUMP008	110/110	110/110	110/110	No
11	CUMP016	157/157	100/100	157/100	Yes
12	CTM9	250/250	250/250	250/250	No
13	ICMP3086	250/250	270/270	250/250	Yes
14	ICMP3088	230/230	210/210	230/210	Yes
15	ICMP3092	175/175	160/160	175/160	Yes
16	ICMP3094	160/160	160/160	160/160	No
17	ICMP4006	320/320	300/300	300/320	Yes
18	ICMP4010	280/280	250/250	280/250	Yes
19	ICMP4012	110/110	90/90	90/110	Yes
20	IPES0035	270/270	270/270	270/270	No

Table3 Allelic profile of parent ICMA04999 (A- Line) and ICMR20233 (R- Line) using 20 SSR primers

S.No.	Primer Name	Observed band size (bp)			Polymorphism
		(A- Line) ICMA04999	(R- Line) ICMR20233	F_1-20574-1	
1	ICMP3088	169/169	194/194	149/169	Yes
2	IPES0019	116/116	109/109	116/116	Yes
3	PSMP2240	185/185	185/185	185/185	No
4	CTM27	190/190	200/200	190/200	Yes
5	IPES0163	190/190	180/180	180/190	Yes
6	ICMP3066	210/210	200/200	210/210	Yes
7	CTM1	150/150	170/170	150/170	Yes
8	CTM10	190/190	210/190	210/190	Yes
9	CTM57	200/200	215/215	200/215	Yes
10	CUMP008	110/110	110/110	110/110	No
11	CUMP016	100/100	120/120	100/100	Yes
12	CTM9	250/250	250/250	250/250	No
13	ICMP3086	250/250	270/270	250/250	Yes
14	ICMP3088	230/230	210/210	230/210	Yes
15	ICMP3092	175/175	160/160	175/160	Yes
16	ICMP3094	160/160	185/185	160/185	Yes
17	ICMP4006	320/320	300/300	300/320	Yes
18	ICMP4010	280/280	250/250	280/250	Yes
19	ICMP4012	110/110	90/90	90/110	Yes
20	IPES0035	270/270	270/270	270/270	No

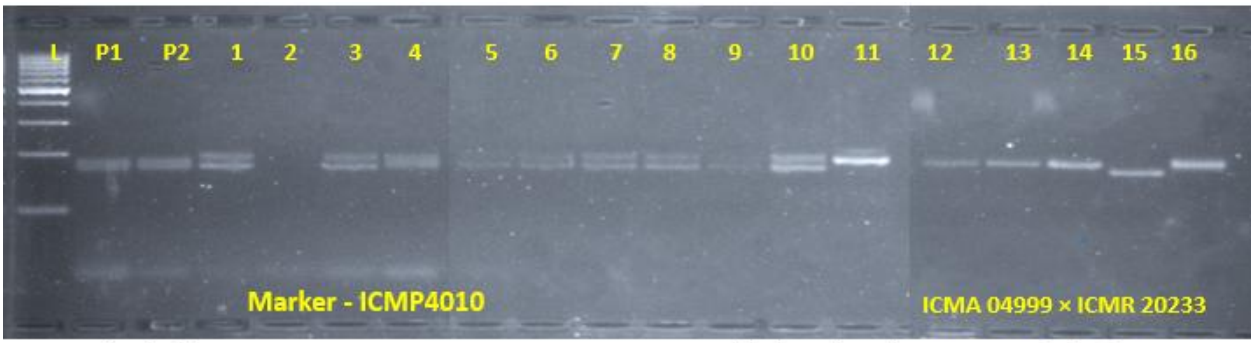
Table4 Allelic profile of parent ICMA02333(A- Line) and ICMR20342 (R- Line) using 20 SSR primers

S.No.	Primer Name	Observed band size (bp)			Polymorphism
		(A- Line) ICMA02333	(R- Line) ICMR20342	F_1-20771-3	
1	ICMP3088	169/169	194/194	149/169	Yes
2	IPES0019	116/116	109/109	116/116	Yes
3	PSMP2240	185/185	185/185	185/185	No
4	CTM27	190/190	200/200	190/200	Yes
5	IPES0163	190/190	180/180	180/190	Yes
6	ICMP3066	210/210	200/200	210/210	Yes
7	CTM1	150/150	170/170	150/170	No
8	CTM10	190/190	210/190	210/190	Yes
9	CTM57	200/200	215/215	200/215	Yes
10	CUMP008	110/110	110/110	110/110	No
11	CUMP016	100/100	120/120	100/100	No
12	CTM9	250/250	250/250	250/250	No
13	ICMP3086	250/250	270/270	250/250	Yes
14	ICMP3088	230/230	210/210	230/210	Yes
15	ICMP3092	175/175	160/160	175/160	Yes
16	ICMP3094	160/160	185/185	160/185	Yes
17	ICMP4006	320/320	300/300	300/320	Yes
18	ICMP4010	280/280	250/250	280/250	Yes
19	ICMP4012	110/110	90/90	90/110	Yes
20	IPES0035	270/270	270/270	270/270	No



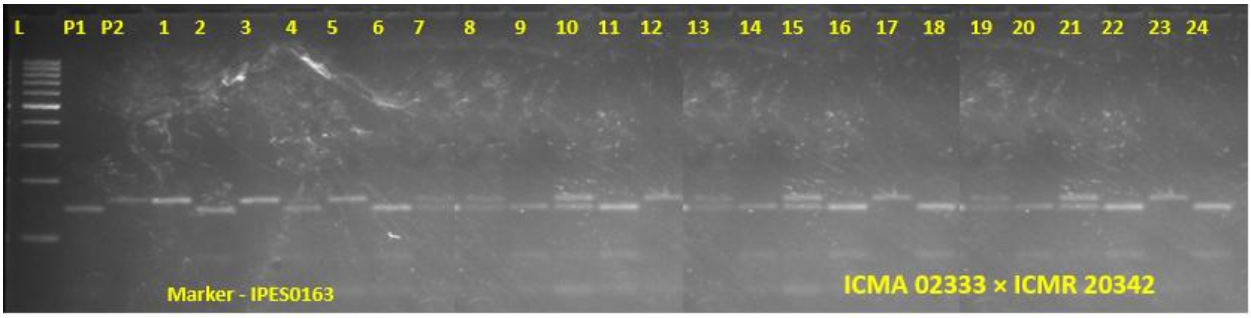
L = 100 bp ladder P1- ICMA 843-22, P2- ICMR 01004, = Double bands indicating true hybrids

Fig. 1 Hybridity test in the cross combinationICMA 843-22 × ICMR 01004



L = 100 bp ladder P1- ICMA 04999 , P2- ICMR 20233 , = Double bands indicating true hybrids

Fig.2Hybridity test in the cross combinationICMA 04999 × ICMR 20233



L = 100 bp ladder P1- ICMA 02333 , P2- ICMR 20342 , = Double bands indicating true hybrids

Fig 3. Hybridity test in the cross combination ICMA 02333 × ICMR 20342