

Bulked segregant analysis to identify SSR markers for loose smut resistance in bread wheat

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ABSTRACT

The present study was conducted to evaluate 100 F₈ recombinant inbred lines (RILs) derived from a cross between resistant and susceptible bread wheat landraces for loose smut caused by *Ustilago tritici*. Bulk segregant analysis (BSA) was also used to identify simple sequence repeats (SSR) markers associated with the resistance to loose smut. The artificial inoculation was repeated twice through two successive seasons (2017/2018 and 2018/2019) under greenhouse conditions, and responses of tested genotypes against loose smut were evaluated in 2018/2019 and 2019/2020 seasons. Highly significant differences ($P < 0.01$) were observed for loose smut incidence among the tested RILs, indicating a considerable genetic variation. On average, loose smut incidence (LSI %) of the tested RILs ranged from 0% in RIL-64 to 97.5% in RIL-74 and RIL-93, with an average of 47.5%. Out of 100 RILs, the RIL-64 was considered as an immune genotype (no symptoms were observed on any plant), RIL-13, RIL-16, RIL-27 and RIL-37 were highly resistant (LSI= 5.0, 5.0, 2.5, 2.5%, respectively), and RIL-2, RIL-50 and RIL-96 were resistant to loose smut (LSI= 7.5, 7.5 and 10.0%, respectively), suggesting the usefulness of these genotypes for developing wheat varieties resistant to loose smut. BSA with forty two SSRs markers identified five positive alleles generated by Xgwm18-1B, Xgwm95-2A, Xgwm294-2A, Xgwm186-5A and Xgwm293-5A markers that were associated with loose smut resistance in the tested population, suggesting that several loci could be contributed to loose smut resistance in wheat. However further studies are still required to confirm usefulness of these markers in breeding programs.

KEYWORDS: Bread wheat, Loose smut, *Ustilago tritici*, Disease resistance, Bulk Segregant Analysis, SSRs.

1. INTRODUCTION

Loose smut of wheat is a seed borne disease caused by the fungus *Ustilago tritici* (Kumar *et al.*, 2018; Chakraborty *et al.*, 2021). It is considered as a common disease in different wheat-growing regions worldwide, causing significant reductions of wheat productivity (Thambugala *et al.*, 2020). In Egypt, loose smut is ranked as the second serious disease infecting wheat following rusts, and it is also considered as a disease of economic important due to its negative effects on grain yield and quality of wheat (Gad *et al.*, 2019). The pathogen remains as a dormant mycelium within the mature seed and germinates with germinating seeds. After seed germination, the mycelium grows in the crown node and later invades inflorescence tissues (Yadav *et al.*, 2020). The pathogens develop systemically in the host plant during growth of the plant, and finally the spike floral tissues of the infected plants are replaced with black masses of teliospores, causing a significant yield reduction (Kumar *et al.*, 2018).

Since loose smut pathogen remains dormant in the mature seed (Yadav *et al.*, 2020), the smut

fungi have been difficult to control with surface fungicide treatments. Therefore, resistant wheat genotypes have been long used as an effective method for loose smut management in wheat (Nielsen, 1983). Furthermore, the development of resistant cultivars is most desirable as an environment-friendly strategy for management of the disease (Menzies, 2008). The infected seed as well as the infected plant cannot be distinguished from a healthy one until the head (spike) starts to emerge. Unlike, loose smut can be easily recognized at the stage of spike emergence as individual grains are completely replaced with masses of black fungal spores (Kumar *et al.*, 2018).

Interestingly, it has been reported that the loose smut resistance in wheat might be inherited as a qualitative or a quantitative trait (Knox *et al.*, 2014). However, different gene actions were reported in wheat, which were depending on the studied varieties and the pathogens (Nielsen and Thomas, 1996; Knox and Menzies, 2012). The additive gene actions were revealed in most cases, and the duplicate complementary action of different

genes was also detected (Syukov and Porotkin, 2015). Multiple genes for loose smut resistance have been previously identified on different wheat chromosomes (Knox *et al.*, 2002; McIntosh *et al.*, 2013; Kassa *et al.*, 2014; Syukov *et al.*, 2015). Different major genes were also found to be located on different wheat chromosomes (Knox and Menzies, 2012). In addition, several quantitative trait loci (QTLs) governing loose smut resistance were previously mapped on different wheat chromosomes by using molecular markers (Toor *et al.*, 2013; Knox *et al.*, 2014; Kumar *et al.*, 2018; Thambugala *et al.*, 2020).

The association between SSR markers and genes controlling loose smut resistance has been reported in wheat (Randhawa *et al.*, 2009; Knox *et al.*, 2014; Kumar *et al.*, 2018; Kumar *et al.*, 2021). Bulk segregant analysis (BSA) developed by Michelmore *et al.* (1991) has been proven as a rapid and efficient method to identify molecular markers for important traits (Torres *et al.*, 2010; Barakat *et al.*, 2011; Hassan *et al.*, 2016). In addition, BSA has been used to identify molecular markers associated with loose smut resistance in wheat (Kassa *et al.*, 2014; Kassa *et al.*, 2015). Since chromosomal locations of numerous SSR markers have been reported in different species, the map location of closely linked QTLs can be determined using BSA without the need for genotyping every individual in the population (Quarrie *et al.*, 1999).

Therefore, in the present study, a population of 100 F₈ recombinant inbred lines (RILs) derived from a cross between two Egyptian bread wheat landraces were evaluated for the resistance to loose smut under greenhouse conditions. The objectives were (1) to assess the responses of tested RILs against loose smut caused by *Ustilago tritici*; (2) to identify resistant bread wheat genotypes to be used in breeding programs; and (3) to identify SSR markers associated with loose smut resistance in the RIL population using BSA.

2. MATERIALS AND METHODS

2.1. Plant material and greenhouse experiments:

The plant material used in this study consisted of 100 F₈ recombinant inbred lines (RILs) of bread wheat (*Triticum aestivum* L.) which derived from a cross between two Egyptian landraces, quite variable in their resistance against loose smut. The artificial infection and evaluation of tested RILs against loose smut were carried out at the greenhouse of Department of Plant Pathology, Faculty of Agriculture, Assiut University, Egypt during three wheat growing seasons. The artificial inoculation of the tested RILs with *U. tritici* was repeated twice through 2017/2018 and 2018/2019

seasons, and evaluating responses of inoculated wheat genotypes against loose smut was carried out during 2018/2019 and 2019/2020 seasons.

2.2. Artificial infection and disease assessment:

The artificial inoculation of tested RILs with *U. tritici* was carried out in the greenhouse following Kumar *et al.* (2018). In brief, twenty seeds of each RIL were sown on a normal sowing date in sterilized pots (25 cm in diameter) containing a peat/sand mixture, following a randomized complete blocks design (RCBD) with four replicates (pots) for each RIL. Syringe technique of spore inoculation was used at the mid-anthesis of wheat plants. A teliospore suspension of *U. tritici* was prepared (1g of teliospores/1L of distilled water). On the morning, all florets on each spike of grown plants were inoculated with the teliospore suspension using a hypodermic needle. The inoculated plants were labeled, and the spikes were harvested at the maturity and the grains were stored for disease assessment in the next season. For disease assessment (next season), twenty seeds of each RIL collected from the previous season were sown on a normal sowing date in sterilized pots following a RCBD with four replicates for each RIL. At the maturity, loose smut incidence (LSI %) of each RIL was determined following Kumar *et al.* (2018) and Thambugala *et al.* (2020) as follows:

$$\text{LSI (\%)} = \frac{\text{Number of smutted plants}}{\text{Total number of plants}} \times 100$$

The reactions of tested RILs against loose smut were assessed based on the disease incidence (Mean LSI, averaged two years) using the rating scale adopted from Ilyas *et al.* (1990) as follows:

- LSI = 0% : Immune (I);
- LSI = 0.1 to < 6% : Highly resistant (HR);
- LSI = 6 to < 11% : Resistant (R);
- LSI = 11 to < 21% : Moderately resistant (MR);
- LSI = 21 to < 31% : Moderately susceptible (MS);
- LSI = 31 to < 51% : Susceptible (S);
- LSI = 51 to 100% : Highly Susceptible (HS);

2.3. Statistical analysis

The data of LSI (%) of the tested RILs in 2018/19 and 2019/20 seasons were subjected to a combined analysis of variance (ANOVA) across the two environments (seasons) to test the significance of differences among genotypes (G) and environments (E) and the significance of G×E interaction. The significance of differences for LSI % was tested using Least Significant Difference (LSD) at 5% probability. The coefficient of variation (%) was calculated. Pearson's correlation coefficient

between LSI of the two years was also estimated.

2.4. Bulked segregant analysis (BSA)

To identify SSR markers associated with loose smut resistance in specific genomic regions, the F₈ RILs population was subjected to BSA (Quarrie *et al.*, 1999) with a total of forty two wheat microsatellites or SSR primer pairs representing all wheat chromosomes of A, B and D genomes of wheat (Table 1). The highly resistant seven RILs as well as the highly susceptible seven RILs were selected from the F₈ RILs population based on mean LSI (averaged two years) and used to construct two DNA bulks for BSA. Genomic DNA extraction was performed using Murray and Thompson (1980) method. Aliquots of DNA from each RIL in each group were mixed to produce resistant and susceptible DNA bulks. Sequences of SSR primers, chromosomal locations and PCR conditions were obtained by the GrainGenes Database (<http://wheat.pw.usda.gov>). PCR amplifications were performed in a SensoQuest LabCycler with OnePCR master mix (GeneDireX, Inc.). PCR products were separated on 2.5% agarose gels in TBE buffer (0.5 X). A 100bp DNA Ladder was used to estimate the size of amplified DNA fragments (bands). Putative polymorphisms between resistant and susceptible bulks were detected for each marker.

3. RESULTS AND DISCUSSION

A total of 100 F₈ RILs of bread wheat were evaluated for their responses against loose smut caused by *Ustilago tritici* under greenhouse conditions. Phenotypic evaluation of loose smut requires two successive seasons; the first season to inoculate tested wheat genotypes with *U. tritici*, and the second to evaluate response of the inoculated plants to the infection (Kumar *et al.*, 2018). In the present study, the artificial inoculation was repeated twice during 2017/2018 and 2018/2019 seasons, and the response of tested RILs were evaluated for two years after each inoculation. Disease symptoms were easily detected at the spike emergence stage as the floral tissues of the infected spike (head) were completely replaced with masses of black spores, as shown in Fig.1. Consequently, the infected plant produces smutted heads instead of grains, and thereby loose smut can cause significant reductions of wheat grain yield (Thambugala *et al.*, 2020). It has been reported that the reduction in grain yield is approximately equal to the percentage of smutted heads on the plant (Gad *et al.*, 2019).

The combined ANOVA across the two environments (seasons) revealed highly significant differences ($P<0.01$) for loose smut incidence (LSI %) among the tested RILs, indicating the presence of a considerable genetic variation. A significant

difference ($P<0.05$) was also observed between the two environments. However, the genotypes-by-environment interaction was non-significant (Table 2). The frequency distribution of LSI (%) in the 10F₈ RILs population in 2018/2019 and 2019/2020 seasons (Fig.2) indicated transgressive segregation of different genes controlling loose smut, as previously reported in wheat (Knox *et al.*, 1999; Kumar *et al.*, 2018).

A highly significant and positive correlation was observed between LSI (%) of the two growing seasons ($r= 0.86$; $P<0.01$). On average, the LSI of the RILs population ranged from 0% in RIL-64 to 97.5% in RIL-74 and RIL-93, with an average of 47.5%. Based on the rating scale described by Ilyas *et al.* (1990), out of 100 RILs evaluated, the RIL-64 was considered as an immune genotype (no symptoms were observed on any plant). In addition, 4 RILs were highly resistant (RIL-13, RIL-16, RIL-27 and RIL-37) and 3 RILs were resistant to loose smut (RIL-2, RIL-50 and RIL-96). In addition, 9 RILs were moderately resistant, 13 RILs were moderately susceptible, 33 RILs were susceptible and 37 RILs were highly susceptible to loose smut (Table 3).

In Egypt, a large number of wheat landraces possess considerable genetic diversity including resistance genes against different plant pathogens. The RILs population evaluated in the present study was derived from a cross between two Egyptian landraces, quite variable in their resistance to loose smut. In this regard, it has been reported that old varieties and local populations or landraces are valuable genetic resources for plant breeders due to their adaptability to marginal environments and higher resistance to pathogens (Pietrusińska *et al.*, 2018). Unlike, the pure genotypes may lack the wide adaptation and the diverse genetic background already present in landraces (Jaradat, 2013). Thus, it is of great importance to identify resistant genetic resources from landraces to be used in wheat breeding programs (Mahmoud *et al.*, 2015). Furthermore, wheat landraces is a potential source of novel resistance genes (Sthapit *et al.*, 2014). Developing resistant wheat cultivars is also considered as the most desirable and environment-friendly strategy for the disease management (Menzies, 2008). Therefore, RILs identified in the present study as immune, highly resistant as well as resistant to loose smut may possess diverse resistance genes, and thereby can be considered as valuable genetic resources for developing disease-resistant bread wheat varieties through breeding programs.

In the present study, the RILs population was subjected to bulked segregant analysis (BSA) to identify SSR markers for loose smut resistance.

Table 1. Names, chromosomal locations, sequences of forward and reverse primers and annealing temperature °C (Tm) of 42 SSR markers used for BSA.

Name	Forward primer (5' - 3')	Reverse primer (5' - 3')	Tm (°C)
Xgwm33-1A	GGAGTCACACTGTTTGTGCA	CACTGCACACCTAACTACCTGC	60
Xgwm497-1A	GTAGTGAAGACAAGGGCATT	CCGAAAAGTTGGGTGATATAC	55
Xgwm95-2A	GATCAAACACACACCCTCC	AATGCAAAGTGAAAAACCCG	60
Xgwm294-2A	GGATTGGAGTTAAGAGAGAACCG	GCAGAGTGATCAATGCCAGA	55
Xgwm339-2A	AATTTTCTTCTCACTTATT	AAACGAACAACCACTCAATC	50
Xgwm356-2A	AGCGTTCTTGGGAATTAGAGA	CCAATCAGCCTGCAACAAC	55
Xgwm155-3A	CAATCATTTCCTCCCTCC	AATCATTGGAAATCCATATGCC	60
Xgwm160-4A	TTCAATTCAGTCTTGGCTTGG	CTGCAGGAAAAAAGTACACCC	55
Xgwm165-4A	TGCAGTGGTCAGATGTTTCC	CTTTTCTTTCAGATTGCGCC	60
Xgwm186-5A	GCAGAGCCTGGTTCAAAAAG	CGCCTCTAGCGAGAGCTATG	60
Xgwm291-5A	CATCCCTACGCCACTCTGC	AATGGTATCTATTCCGACCCG	60
Xgwm292-5A	TCACCGTGGTCACCGAC	CCACCGAGCCGATAATGTAC	60
Xgwm293-5A	TACTGGTTCACATTGGTGCG	TCGCCATCACTCGTTCAAG	55
Xbarc186-5A	GGAGTGTGAGATGATGTGGAAC	CGCAGACGTCAGCAGCTCGAGAGG	60
Xgwm459-6A	ATGGAGTGGTCACACTTTGAA	AGCTTCTCTGACCAACTTCTCG	55
Xbarc113-6A	GCGCACAACAACGGACACTTAACAAT	GGGACTCATTAGCTTCTACTCGCCATTA	50
Xgwm63-7A	TCGACCTGATCGCCCCTA	CGCCCTGGGTGATGAATAGT	60
Xwmc273-7A	AGTTATGTATTCTCTCGAGCCTG	GGTAACCACTAGAGTATGTCCTT	50
Xwmc596-7A	TCAGCAACAAACATGCTCGG	CCCGTGTAGGCGGTAGCTCTT	60
Xwmc603-7A	ACAAACGGTGACAATGCAAGGA	CGCCTCTCTCGTAAGCCTCAAC	60
Xbarc121-7A	ACTGATCAGCAATGTCAACTGAA	CCGGTGTCTTTCCTAACGCTATG	50
Xgwm18-1B	GGTTGCTGAAGAACCTTATTTAGG	TGGCGCCATGATTGCATTATCTTC	50
Xgwm111-2B	GTTGCACGACCTACAAAGCA	ATCGCTCACTACTATCGGG	55
Xgwm389-3B	ATCATGTCGATCTCCTTGACG	TGCCATGCACATTAGCAGAT	60
Xgwm493-3B	TTCCATAACTAAAACCGCG	GGAACATCATTTCTGGACTTTG	60
Xgwm533-3B	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGAAAAGCC	60
Xgwm566-3B	TCTGTCTACCCATGGGATTG	CTGGCTTCGAGGTAAGCAAC	60
Xgwm113-4B	ATTCGAGGTTAGGAGGAAGAGG	GAGGGTCGGCCTATAAGACC	55
Xgwm513-4B	ATCCGTAGCACCTACTGGTCA	GGTCTGTTTCATGCCACATTG	60
Xgwm408-5B	TCGATTTATTTGGGCCACTG	GTATAATTCGTTACAGCACGC	55
Xgwm626-6B	GATCTAAAATGTTATTTTCTCTC	TGACTATCAGCTAAACGTGT	50
Xwmc398-6B	GGAGATTGACCGAGTGGAT	CGTGAGAGCGGTTCTTTG	60
Xgwm577-7B	ATGGCATAATTTGGTGAAATTG	TGTTTCAAGCCCAACTTCTATT	55
Xgwm458-1D	TTGCAATGTTGATTTGGC	TTGCAATGTTGATTTGGC	60
Xbarc229-1D	GGCCGCTGGGATTGCTATGAT	TCGGGATAAGGCAGACCACAT	55
Xgwm261-2D	CTCCCTGTACGCCTAAGGC	CTCGCGCTACTAGCCATTG	55
Xgwm484-2D	ACATCGCTCTCACAAACCC	AGTTCCGGTCATGGCTAGG	55
Xwmc601-2D	ACAGAGGCATATGCAAAGGAGG	CTTGTCTCTTTATCGAGGGTGG	60
Xgwm3-3D	AATATCGCATCACTATCCCA	AATATCGCATCACTATCCCA	55
Xgwm190-5D	GTGCTTGCTGAGCTATGAGTC	GTGCCACGTGGTACCTTTG	60
Xgwm325-6D	TTTCTTCTGTCGTTCTCTCC	TTTTTACGCGTCAACGACG	60
Xgwm437-7D	GATCAAGACTTTTGTATCTCTC	GATGTCCAACAGTTAGCTTA	50

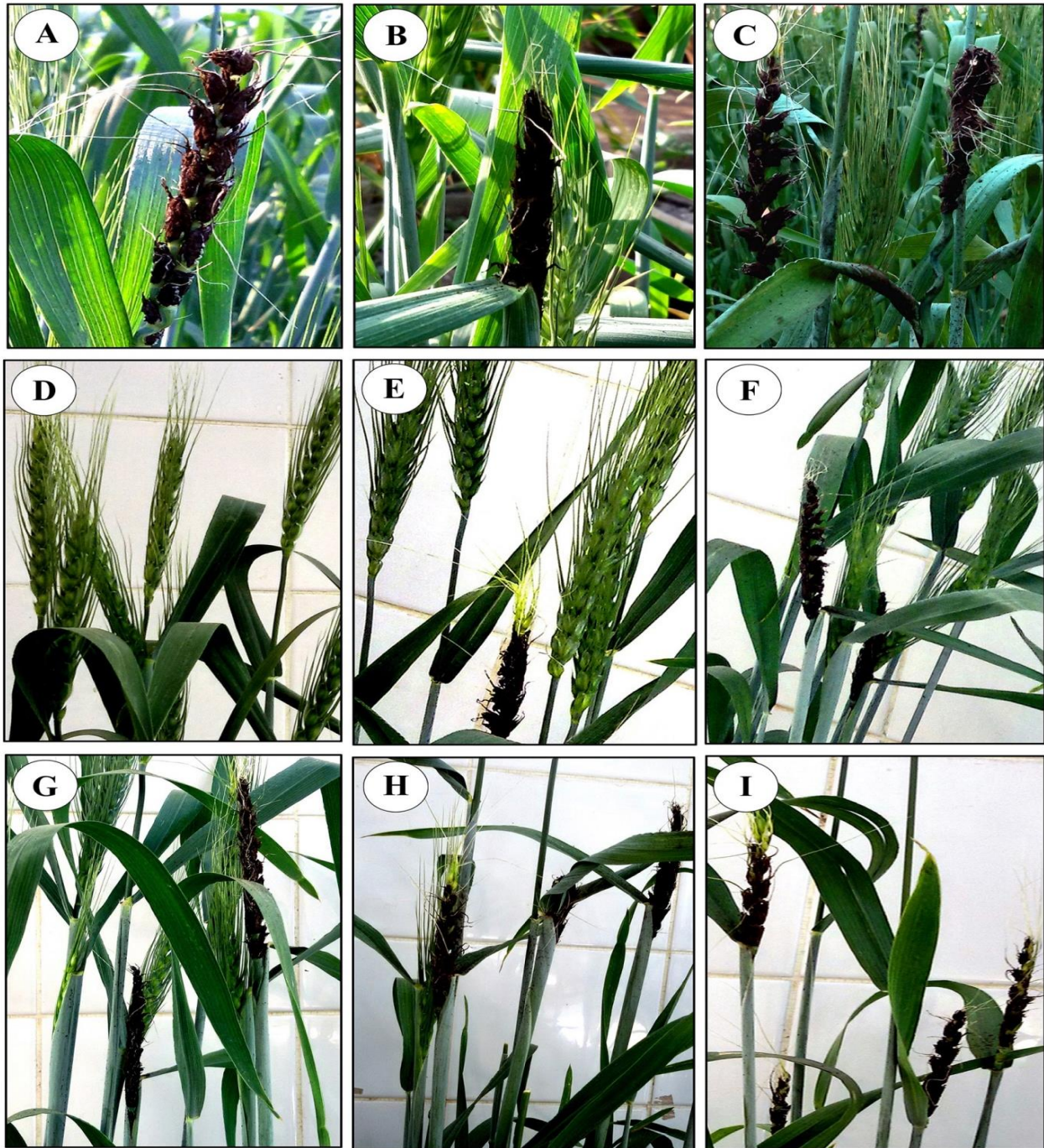


Fig.1. Loose smut symptoms caused by *Ustilago tritici* on different RILs of bread wheat: (A, B and C) show floral parts of different wheat spikes that were completely replaced by masses of black teliospores, (D) shows a RIL with no symptoms on any plant (immune), and (E to I) show RILs with difference loose smut incidence.

Table 2. The combined ANOVA of loose smut incidence across two environments (2018/2019 and 2019/2020 seasons) in 100 RILs population of bread wheat.

Source of variance	d.f	Sum of squares	Mean square
Environments (E)	1	1922.0	1922.0*
Replicates within E	6	1268.0	211.3
Genotypes (G)	99	631422.0	6378.0**
G×E interaction	99	43478.0	439.2
Pooled error	594	278932.0	469.6

* and ** indicate significant differences at 0.05 and 0.01 probability.

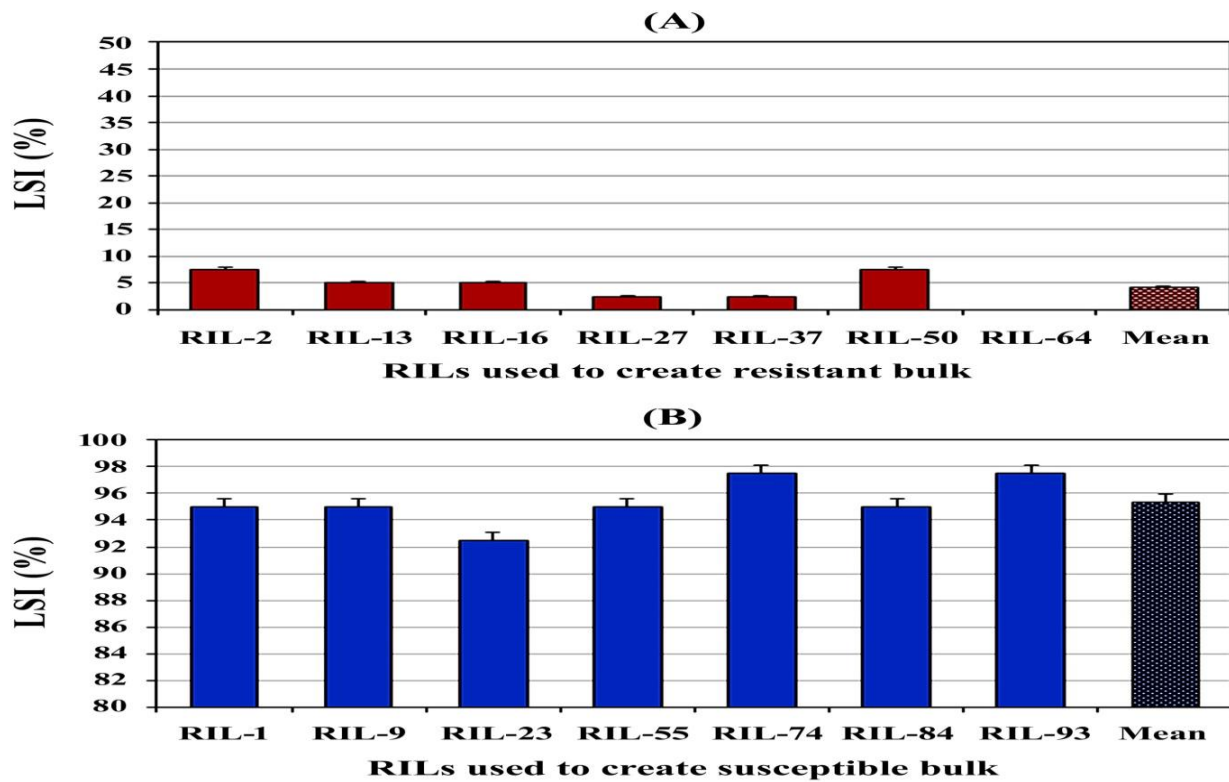


Fig.2. Loose smut incidence (LSI %) of RILs used to create resistant (A) and susceptible (B) bulks for BSA.

The immune and highly resistant seven RILs showing the lowest LSI (%) as well as the highly susceptible seven RILs showing the highest LSI (%) averaging two years were used to construct two DNA bulks for BSA. Mean LSI of the RILs used to construct the resistant bulk; namely RIL-2, RIL-13, RIL-16, RIL-27, RIL-37, RIL-50 and RIL-64 were 7.5, 5.0, 5.0, 2.5, 2.5, 7.5 and 0%, respectively, with an average of 4.3%. Meanwhile, mean LSI of the RILs used to construct the susceptible bulk; namely RIL-1, RIL-9, RIL-23, RIL-55, RIL-74, RIL-84 and RIL-93 were 95.0, 95.0, 92.5, 95.0, 97.5, 95.0 and 97.5%, respectively, with an average of 95.4% (Table 3; Fig.3).

The PCR assays with SSR markers generated different number of bands (alleles) which were depending on the primers used, indicating the presence of allelic diversity among SSRs (Ravi *et al.*, 2003; Ram *et al.*, 2007). It has been reported that such variation in the allelic diversity is due to several factors including the structure of primers used and number of annealing sites in the genome (Muralidharan and Wakeland, 1993).

Out of forty two SSRs markers used with BSA, five SSRs generated nine polymorphic bands, which were able to distinguish the resistant from susceptible bulk (Fig.4 to Fig.6). Of which, five positive bands (alleles) generated by the markers Xgwm18-1B (192 bp), Xgwm95-2A (160 bp), Xgwm294-2A (350 bp), Xgwm186-5A (188 bp) and Xgwm293-5A (94 bp) were associated with the

resistance RILs, while four alleles generated by the markers Xgwm95-2A (146 bp), Xgwm294-2A (310 bp), Xgwm186-5A (200 bp) and Xgwm293-5A (118 bp) were associated with susceptible RILs (Table 4). In accordance, BSA has been used by Kassa *et al.* (2014) and Kassa *et al.* (2015) to identify molecular markers for loose smut resistance in wheat. Furthermore, BSA has been widely used to identify SSR markers associated with important traits (Torres *et al.*, 2010; Barakat *et al.*, 2011; Hassan *et al.*, 2016; Mahmoud and Hassan, 2020). The association between SSR markers and genes or QTLs controlling loose smut resistance has been also reported in wheat (Randhawa *et al.*, 2009; Knox *et al.*, 2014; Kumar *et al.*, 2018). Multiple genes controlling loose smut resistance have been previously identified in wheat on different wheat chromosomes (Knox *et al.*, 2002; McIntosh *et al.*, 2013; Kassa *et al.*, 2014; Syukov *et al.*, 2015). Of which, Ut1 and Ut2 were found to be located on chromosome 6A, Ut3 and Ut4 on 7B, Ut5 on 2B, Ut6 on 5B, Ut7 on 7A, Ut8 on 3A, Ut9 on 6B and Ut10 on 6D (Knox and Menzies, 2012; McIntosh *et al.*, 2013; Kassa *et al.*, 2014; Kassa *et al.*, 2015; Knox *et al.*, 2014; Syukov *et al.*, 2015; Serfling *et al.*, 2017). In addition, different QTLs governing loose smut resistance were previously mapped by using molecular markers on chromosomes 1B, 3A, 3B, 4B, 5B, 6A, 6B, 6D, 7A and 7B of wheat (Toor *et al.*, 2013; Knox *et al.*, 2014; Kumar *et al.*, 2018; Thambugala *et al.*, 2020).

Table 3. Response of 100 F₃ RILs against loose smut (averaged 2018/2019 and 2019/2020 seasons).

No.	LSI (%)	Reaction	No.	LSI (%)	Reaction	No.	LSI (%)	Reaction
1	95.0	HS	35	35.0	S	69	35.0	S
2	7.5	R	36	75.0	HS	70	32.5	S
3	27.5	MS	37	2.5	HR	71	82.5	HS
4	40.0	S	38	77.5	HS	72	25.0	MS
5	80.0	HS	39	20.0	MR	73	65.0	HS
6	35.0	S	40	12.5	MR	74	97.5	HS
7	80.0	HS	41	80.0	HS	75	92.5	HS
8	80.0	HS	42	60.0	HS	76	45.0	S
9	95.0	HS	43	45.0	S	77	40.0	S
10	90.0	HS	44	32.5	S	78	80.0	HS
11	77.5	HS	45	32.5	S	79	22.5	MS
12	17.5	MR	46	37.5	S	80	92.5	HS
13	5.0	HR	47	30.0	MS	81	27.5	MS
14	25.0	MS	48	42.5	S	82	25.0	MS
15	15.0	MR	49	45.0	S	83	52.5	HS
16	5.0	HR	50	7.5	R	84	95.0	HS
17	12.5	MR	51	75.0	HS	85	57.5	HS
18	30.0	MS	52	37.5	S	86	25.0	MS
19	27.5	MS	53	30.0	MS	87	15.0	MR
20	32.5	S	54	27.5	MS	88	50.0	S
21	85.0	HS	55	95.0	HS	89	32.5	S
22	85.0	HS	56	35.0	S	90	17.5	MR
23	92.5	HS	57	25.0	MS	91	15.0	MR
24	35.0	S	58	60.0	HS	92	92.5	HS
25	32.5	S	59	32.5	S	93	97.5	HS
26	40.0	S	60	47.5	S	94	65.0	HS
27	2.5	HR	61	37.5	S	95	70.0	HS
28	67.5	HS	62	37.5	S	96	10.0	R
29	32.5	S	63	32.5	S	97	80.0	HS
30	37.5	S	64	0.0	I	98	40.0	S
31	87.5	HS	65	67.5	HS	99	40.0	S
32	47.5	S	66	90.0	HS	100	15.0	MR
33	62.5	HS	67	42.5	S	-	-	-
34	77.5	HS	68	42.5	S	-	-	-

Mean LSI = 47.5%

LSD_(0.05) = 6.0

Coefficient of Variation = 59.0%

In conclusion, the immune, highly resistant and resistant RILs identified in the current study may possess diverse resistance genes, and thereby could be used for developing wheat varieties resistant to loose smut in breeding programs. BSA detected five SSR markers located on different wheat chromosomes that could be considered as markers associated with loose smut resistance in the tested population, suggesting that different loci could be contributed to the loose smut resistance in wheat. However, further studies are required to confirm the utility of SSRs markers identified in the

study in marker-assisted selection (MAS) for loose smut resistance.

4. REFERENCES

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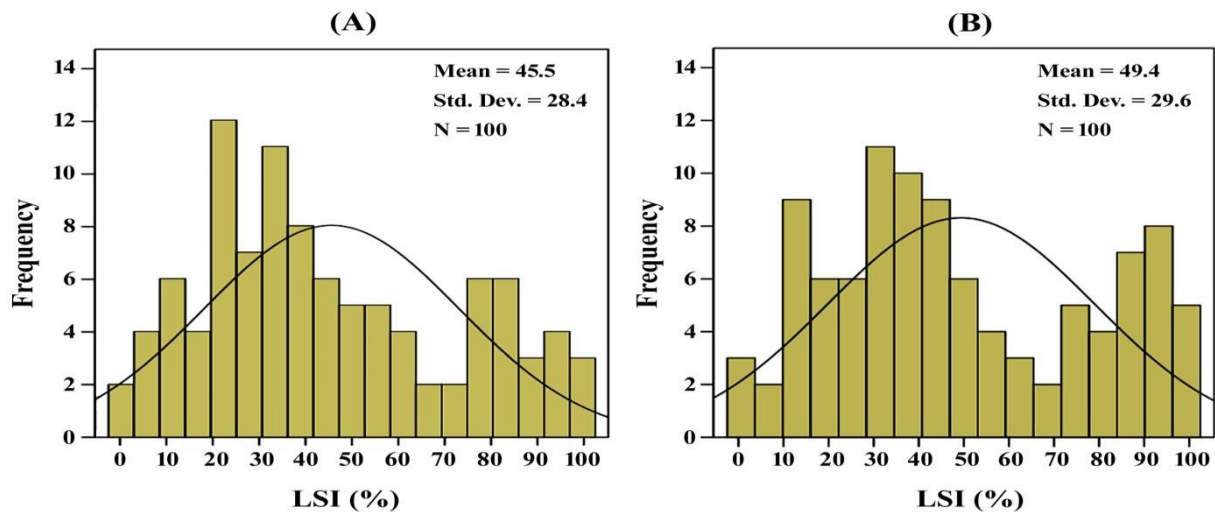


Fig.3. Frequency distribution of LSI (%) in the RILs population in 2018/2019 (A) and 2019/2020 (B) seasons.

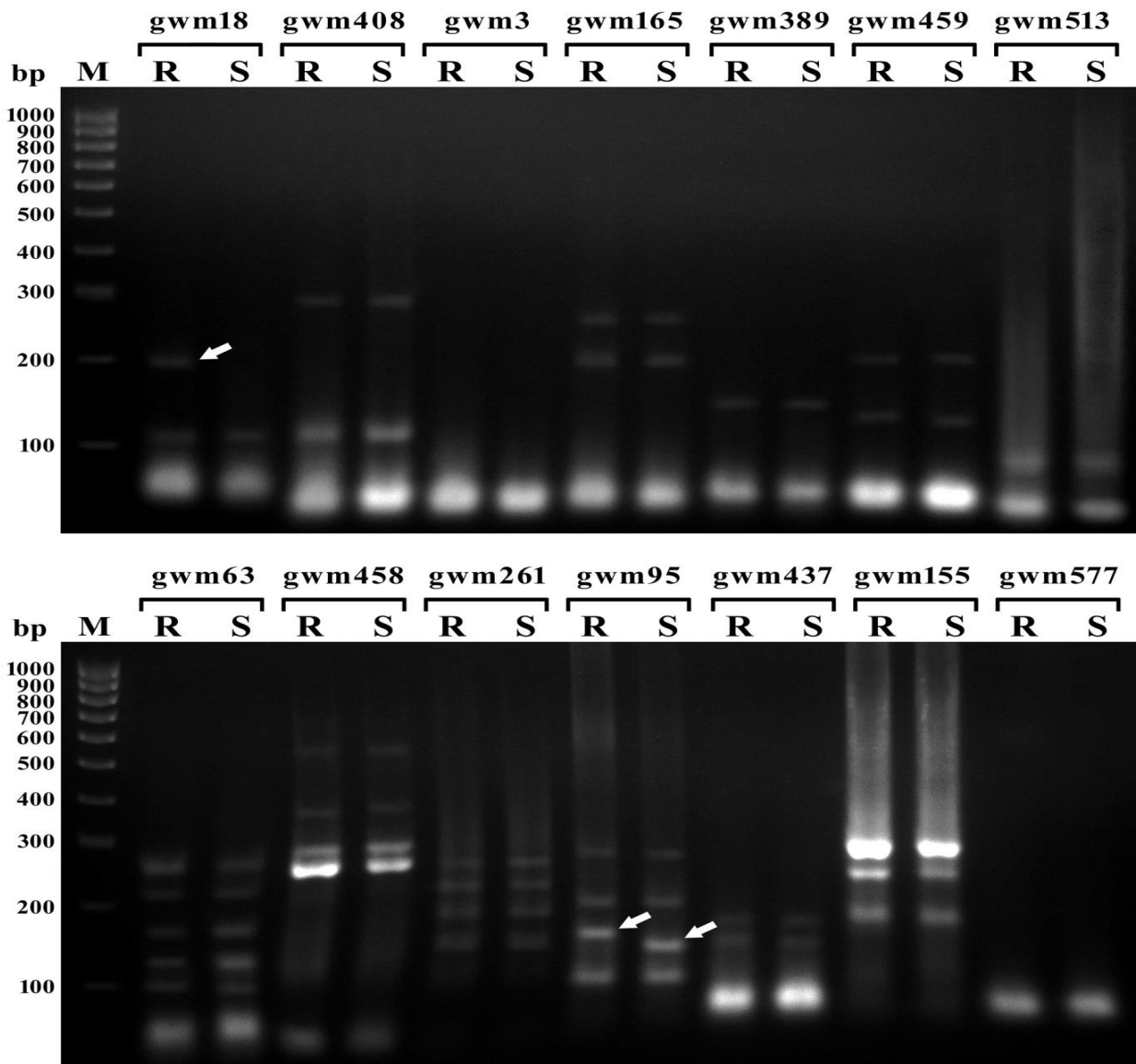


Fig.4. PCR amplifications obtained using BSA with SSR markers. M: A 100bp DNA ladder, R: Resistant bulk and S: susceptible bulk. Polymorphic bands were generated by the markers Xgwm18-1B and Xgwm95-2A. Arrows indicate polymorphic bands (alleles), which distinguished resistant from susceptible bulk.

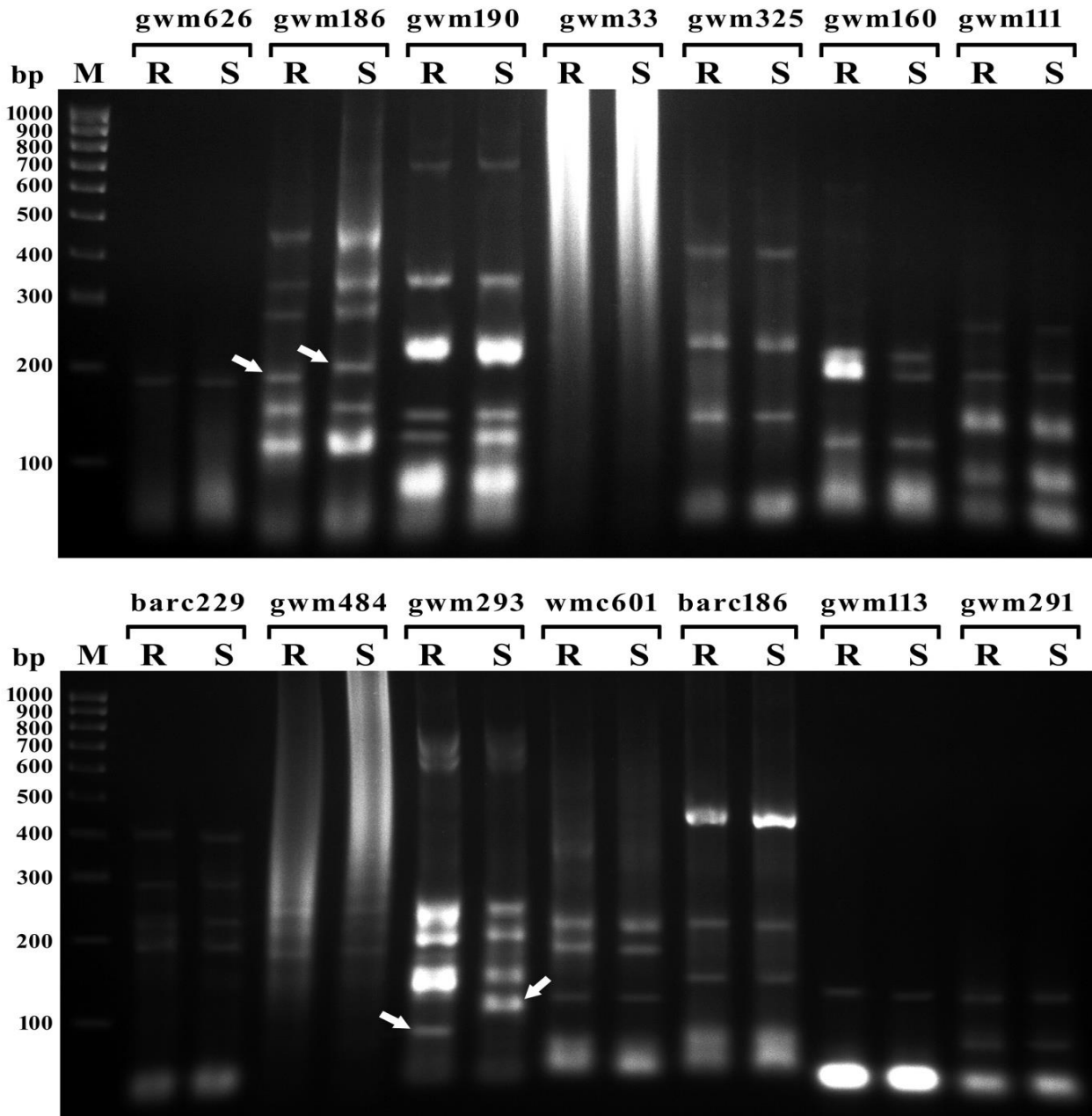


Fig.5. PCR amplifications obtained using BSA with SSR markers. M: A 100bp DNA ladder, R: Resistant bulk and S: susceptible bulk. Polymorphic bands were generated by the markers Xgwm186-5A and Xgwm293-5A. Arrows indicate polymorphic bands (alleles), which distinguished resistant from susceptible bulk.

Table 4. Bands (alleles) detected for resistant and susceptible RILs using BSA.

Marker	Chromosomal location	Size of positive alleles (bp)	
		Resistant	Susceptible
Xgwm18	1B	(+) 192	-
Xgwm95	2A	(+) 160	(+) 146
Xgwm294	2A	(+) 350	(+) 310
Xgwm186	5A	(+) 188	(+) 200
Xgwm293	5A	(+) 94	(+) 118

(+) indicates a presence of a specific band (positive allele) followed by its size (bp).

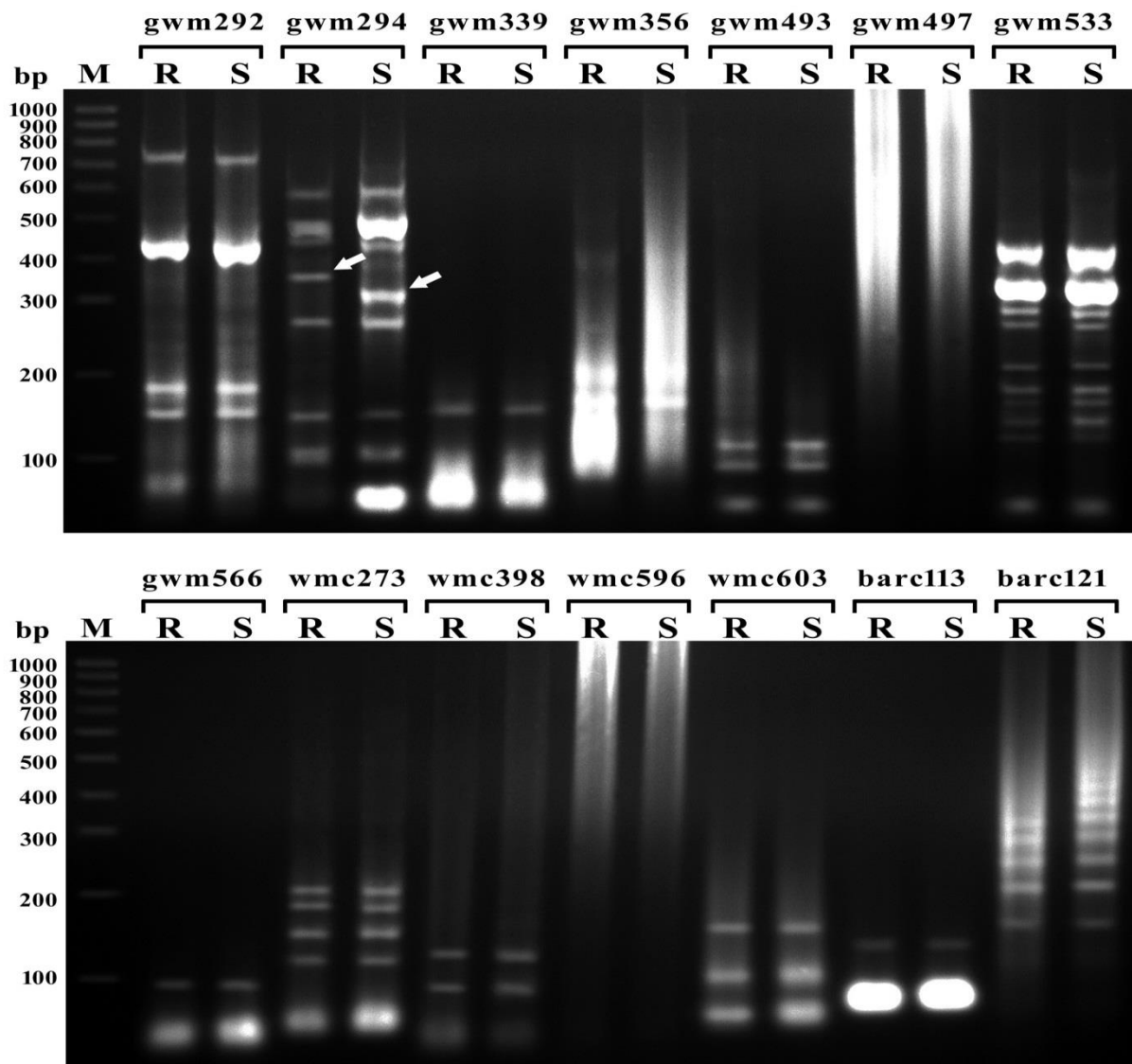


Fig.6. PCR amplifications obtained using BSA with SSR markers. M: A 100bp DNA ladder, R: Resistant bulk and S: susceptible bulk. Polymorphic bands were generated by the marker Xgwm294-2A. Arrows indicate polymorphic bands (alleles), which distinguished resistant from susceptible bulk.

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الملخص العربي

تحليل ضم الإنعزالات المتفارقة لتحديد واسمات جزيئية SSR لمقاومة التفحم السائب في قمح الخبز

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أجريت الدراسة الحالية بهدف تقييم مائة سلالة مربية داخلياً ذات اتحادات وراثية جديدة في الجيل الثامن ناتجة من التهجين بين سلالتين أرضيتين من قمح الخبز إحداهما مقاومة والأخرى حساسة لمرض التفحم السائب الناتج عن الفطر *Ustilago tritici*. تم أيضاً استخدام تحليل ضم الإنعزالات المتفارقة بهدف تحديد بعض واسمات التتابعات البسيطة المتكررة (SSR) مرتبطة بالمقاومة لمرض التفحم السائب. تم تكرار العدوى الإصطناعية بالفطر المسبب للمرض خلال موسمين متتاليين (٢٠١٧/٢٠١٨ و ٢٠١٨/٢٠١٩) تحت ظروف الصوبة، وتم تقييم استجابات الطرز الوراثية المختبرة ضد التفحم السائب في موسمي ٢٠١٨/٢٠١٩ و ٢٠١٩/٢٠٢٠. هذا وقد لوحظت اختلافات معنوية جداً ($P < 0.01$) في نسبة حدوث الإصابة بالتفحم السائب بين السلالات المختبرة، مما يشير إلى وجود تباين وراثي كبير. أظهرت النتائج أنه في المتوسط، تراوحت نسبة حدوث الإصابة بالتفحم السائب في السلالات المختبرة من ٠% في السلالة RIL-64 إلى ٩٧.٥% في السلالتين RIL-74 و RIL-93، بمتوسط قدره ٤٧.٥%. كما لوحظ أنه من بين ١٠٠ سلالة مختبرة، أن السلالة RIL-64 تعتبر كطرز وراثي منيع (لم يلاحظ أي أعراض على أي نبات)، وكانت السلالات RIL-13 و RIL-16 و RIL-27 و RIL-37 شديد المقاومة (نسبة حدوث الإصابة ٥، ٥، ٢.٥، ٢.٥%، على التوالي)، وكانت السلالات RIL-2 و RIL-50 و RIL-96 مقاومة للتفحم السائب (نسبة حدوث الإصابة ٥، ٧.٥، ١٠%، على التوالي)، مما يشير إلى أهمية تلك الطرز الوراثية لتطوير أصناف قمح مقاومة لمرض التفحم السائب. كما أوضحت النتائج نجاح تحليل ضم الإنعزالات المتفارقة باستخدام اثنين وأربعين من واسمات التتابعات البسيطة المتكررة (SSR) في تحديد خمس أليلات نتجت بواسطة الواسمات Xgwm18-1B و Xgwm95-2A و Xgwm294-2A و Xgwm186-5A و Xgwm293-5A كانت مرتبطة بالمقاومة للتفحم السائب في العشيرة المختبرة، مما يشير إلى وجود عدة مواقع وراثية قد تساهم في مقاومة التفحم السائب في القمح. ومع ذلك، لا تزال هناك حاجة إلى مزيد من الدراسات لتأكيد فائدة تلك الواسمات الجزيئية في برامج التربية.