Validation of microsatellite molecular markers linked with resistance to Bipolaris sorokiniana in wheat (Triticum aestivum L.)

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SUMMARY

Spot blotch disease caused by Bipolaris sorokiniana (Sacc.) Shoem causes yield losses and reduces grain quality in wheat. Molecular markers reported to be linked with resistance to B. sorokiniana could accelerate the identification of resistant genotypes as they are independent of the environmental effect. However, before they can be utilized for marker assisted selection (MAS), validation in an independent population is required. The objective of the present study was therefore to validate three simple sequence repeat (SSR) molecular markers (Xwgm570, Xgwm544 and Xgwm437) linked with resistance to B. sorokiniana. The markers were validated using 66 wheat genotypes comprising 11 parental genotypes and 55 F2 progenies. Single marker analysis was performed using simple linear regression to ascertain the relationship between the marker and the trait. All the markers were confirmed to be associated with resistance. They all gave significant association with resistance to B. sorokiniana. The markers amplified DNA fragments in the resistant parental genotypes that were similar to those observed in resistant F2 progenies, but absent in the susceptible ones. Hence, these markers could be useful in increasing the efficiency of selection for resistance to B. sorokiniana in wheat breeding. Since the R2 values are low, a combination of two or three SSR markers can be employed during MAS. This was evident by the multiple linear equation which gave a combined R2 value of 18.0%, obtained from the inclusion of all three markers.

INTRODUCTION

Bipolaris sorokiniana (Sacc.) Shoem is a pathogen that causes spot blotch, a foliar fungal disease in wheat. The disease is more prevalent in warmer and humid environments worldwide, causing significant yield losses and reducing grain quality (Acharya et al. 2011). Utilization of resistant genotypes to B. sorokiniana is the best approach to manage the disease because it is economical and environmentally friendly. However, breeding for resistance has been slow due to ineffectiveness of selection methods (Duveiller et al. 2007). Selection of resistant genotypes has been by screening wheat genotypes in several environments, ‘hot spot’ sites, by use of disease phenotyping to identify resistance sources in order to develop new genotypes with improved resistance (Duveiller & Sharma 2012). Phenotypic markers such as leaf angle, leaf tip necrosis and stay green have been used as an aid to selection for resistance to B. sorokiniana in wheat (Joshi & Chand 2002; Joshi et al. 2004, 2007). The problems associated with phenotypic evaluation of genotypes is that it requires large sample sizes for screening, it is time consuming and laborious, relies greatly on repeated ratings in diverse environments, confounded by environmental factors and incurs high experimental errors (Fernando et al. 2015). Nonetheless, molecular markers linked to B. sorokiniana resistance genes can provide an alternative approach to overcome these drawbacks and accelerate identification and development of resistant genotypes (Concibido et al.)
Quantitative trait loci (QTLs) and simple sequence repeat markers (SSR) linked with resistance to *B. sorokiniana* have been identified in wheat. The identification of QTL and SSR markers associated with resistance to *B. sorokiniana* presented new opportunities for improving resistance in wheat. With utilization of molecular marker-assisted selection (MAS), screening and identification of resistant genotypes in parental and segregating generations could be done in the absence of disease epiphytotic conditions (Mondal et al. 2008). Kumar et al. (2009) identified four QTLs linked to *B. sorokiniana* resistance genes on chromosomes 2A, 2B, 5B and 6D in Yangmai 6, a resistant parent. Two QTLs located on chromosome 2B and 5B were found to be consistent in a 3-year trial. Awasthi & Lal (2014) indicated that a QTL or a molecular marker that is present in numerous genetic backgrounds is important for marker-assisted breeding. Kumar et al. (2010) also identified four QTLs on chromosomes 2AS, 2BS, 5BL and 7DS to be linked with resistance to *B. sorokiniana*. Nonetheless, two markers, Xgwm148 and Xgwm111 closely linked to the QTLs on chromosomes 2B and 7D, respectively, were identified as diagnostic markers for resistance to *B. sorokiniana*. Kumar et al. (2005) reported two SSR markers on chromosome 7D (Xgwm437) and on chromosome 5B (Xgwm544) linked with resistance to *B. sorokiniana* in Yangmai 6. Additionally, three SSR markers linked to *B. sorokiniana* resistance were identified in wheat genotype ‘G162’ on chromosomes 5B (Xgwm67), 6A (Xgwm570) and on 6D (Xgwm469) (Sharma et al. 2007). Adhikari et al. (2012) using Diversity Arrays Technology (DArT) markers identified four genomic regions (chromosomes 1A, 3B, 7B and 7D) associated with resistance to *B. sorokiniana* at the seedling stage.

Validating of molecular markers with another population is required before utilizing them for marker-assisted breeding to determine their effectiveness (Anitha et al. 2014). It is not known whether any previous studies have been conducted to validate SSR markers linked with resistance to *B. sorokiniana*. Validation establishes the value of a molecular marker reported to be linked to a particular trait in an independent population with varying genetic background (Sharp et al. 2001; Islam et al. 2011). The present study was carried out to validate Xgwm544, Xgwm437 and Xgwm570 SSR molecular markers for their association with resistance to *B. sorokiniana* for probable use in marker-assisted breeding.

**MATERIALS AND METHODS**

**Plant material**

A total of 66 wheat genotypes comprising 11 parental genotypes and 55 F₂ lines were used in screening for resistance to *B. sorokiniana*. The 11 parental genotypes included three adapted genotypes from Zambia (Coucal, Kvale and Loerrie II) and the rest were introduced from the International Maize and Wheat Improvement Centre (CIMMYT) – Mexico. The 55 F₂ progenies were derived from the diallel crosses involving eight parents: Coucal, Kvale, Loerrie II, 30SAWSN5, 30SAWSN10, 30SAWSN18, SB50 and 19HRWSN15. SB50 (Sonalia) was used as a susceptible control, while 19HRWSN6 and 30SAWSN5 were used as resistant controls.

**Phenotypic evaluation**

Screening for resistance of the 66 wheat genotypes was done under natural epidemics in three sites in Zambia, Mpongwe Seed-Co Research Farm (12°06′S, 28°15′E, 1221 m a.s.l.), Golden Valley Agricultural Research Trust (GART) (14°58′S and 28°05′E, 1148 m a.s.l.) and Mt. Makulu Research Station (13°32′S and 28°03′E, 1216 m a.s.l.) during the 2013/14 season. The experimental field was laid out in a 6 × 11 alpha lattice design with two replications. Each genotype was planted in a 3 m long plot of two rows with an inter-row spacing of 20 and 40 cm between plots. Each row was seeded with 60 plants, which were later thinned out to 30 plants per row. Standard agronomic practices were followed for good crop management. All plants were scored for disease severity on symptom appearance, at 7-day intervals. The disease severity score was based on Saari and Prescott’s 0–9 scale for assessing foliar disease (Eyal et al. 1987), where zero = no symptoms, 1 = one or two necrotic spots, 2 = a few small necrotic spots without chlorosis, 3 = a few small necrotic spots without chlorosis.
with chlorosis, 4 = medium size necrotic spots with distinct but restricted chlorotic margin, 5 = medium to large necrotic spots with distinct but restricted chlorotic margin, 6 = large abundant necrotic spots with distinct chlorotic margin, 7 = necrotic spots linked together with pronounced chlorosis, 8 = extensive necrotic spots fully merge expanding longitudinally with pronounced chlorosis and 9 = many extensive necrotic spots with pronounced chlorosis. The genotypes were classified on a 9-point scale using the resistance criterion proposed by Chaurasia et al. (1999). Genotypes falling in the 1–3 categories were recorded as resistant, 4 as moderately resistant, 5–6 moderately susceptible and 7–9 as susceptible.

Genotypic evaluation

Genomic DNA extraction

For each genotype, DNA was extracted according to an International Maize and Wheat Improvement Centre protocol (CIMMYT 2005). Twenty seeds for each genotype were ground separately into a fine powder using a pestle and mortar. About 70 mg of seed meal was transferred to 1·5 ml centrifuge tubes and labelled. The extraction buffer of 700 µl (1% SDS (sodium dodecyl sulphate), 700 mM NaCl (sodium chloride), 100 mM Tris (Tris (hydroxymethyl) amino-methane and 50 mM EDTA (ethylenediaminetetra acetate)) was added to the meal and incubated for 10 min at 65 °C. After incubation, 200 µl of 5 M potassium acetate was added to the mixture. The mixture was then vortex and placed on ice for 10 min, before being centrifuged at 12 000 rpm for 10 min. The supernatant was discarded and the DNA pellet washed twice by adding 500 µl ethanol and centrifuging at 8000 rpm for 3 min for each wash. The pellet was dried at room temperature and re-suspended in 50–100 µl of deionized water.

Genotyping with simple sequence repeat markers

The markers Xgwm570 (Sharma et al. 2007), Xgwm544 (Kumar et al. 2005) and Xgwm437 (Kumar et al. 2005) previously reported to be linked to genes for resistance to B. sorokiniana were used in the present study. Primer sequences (Table 1) were obtained from the wheat database GrainGenes, available at http://wheat.pw.usda.gov.

To amplify regions of genomic DNA, polymerase chain reaction (PCR) was performed in a 12 µl volume reaction mixture containing 2 µl of template DNA (10 ng/µl), 1·2 µl deoxynucleoside 5′-triposphates (dNTPs) (25 U/µl), 0·72 µl magnesium chloride (MgCl₂) (50 mM), 1·2 µl buffer (10×), 0·12 µl Taq polymerase (5 U/µl), 6·16 µl PCR grade water, 0·24 µl dye (10 µM), 0·06 µl forward primer and 0·3 reverse primer. The amplification reactions were performed in a heated lid thermal cycle programmed at 93 °C for 1 min for one cycle, followed by 30 cycles of denaturing at 93 °C for 30 s, primer annealing at 60 °C for 30 s and an extension of one cycle of 72 °C for 5 min, followed by a final extension at 72 °C for 10 min. The PCR products were fluorescently labelled and separated by capillary electrophoresis on an ABI 3130 automatic sequencer (Applied Biosystems, Johannesburg, South Africa). Amplification products were scored using GeneMapper 4·1 (CIMMYT 2005).

DATA ANALYSIS

The phenotypic data were subjected to ANOVA (analysis of variance), using the general linear model procedure (PROC GLM) in SAS version 9·3 (SAS Institute 2011), to determine whether there were significant differences among genotypes.

Single marker analysis using regression analysis in SAS version 9·3 (SAS Institute 2011) was done to determine the association between field spot blotch resistance values and the marker genotype data. The band amplified from each marker data was scored as either 0 to indicate absence of the marker or 1 to indicate presence of the marker for regression analysis (Kuldeep et al. 2008; Francis et al. 2011). Significance of the regression coefficient suggests that there is a relationship between the marker and the trait (Anandhan et al. 2010). The single marker analysis was conducted following the linear model below:

\[ Y = a + bX + \text{error} \]

where \( Y \) = trait value, \( a \) = constant, \( b \) = slope or regression coefficient of \( X \) and \( X \) = molecular marker.

A multiple linear regression analysis was conducted in Genstat version 14 (Payne et al. 2011) to investigate effectiveness of using three markers together in marker assisted breeding. The following linear model was...
used for multiple linear regression analysis:

\[ Y = a + b_1X_1 + b_2X_2 + b_3X_3 + \text{error} \]

where \( Y \) = trait value, \( a \) = constant, \( b_1 \) = slope or regression coefficient of \( X_1 \), \( X_1 \) = marker \( Xgwm570 \), \( b_2 \) = slope or regression coefficient of \( X_2 \), \( X_2 \) = marker \( Xgwm544 \) and \( b_3 \) = slope or regression coefficient of \( X_3 \), \( X_3 \) = marker \( Xgwm437 \).

RESULTS

Phenotypic evaluation

Significant differences \((P<0.001)\) were observed among genotypic reaction to spot blotch disease (Table 2). Among the 11 parental genotypes, only one (19HRWSN6) was recorded as moderate resistance (score of 4), six as moderately susceptible (Coucal, SB1, 19HRWSN19, 30SAWSN18, 30SAWSN5, 30SAWSN10) (score of 5–6) and four as susceptible (SB50, 19HRWSN15, Kwale and Loerrie II) (score of 7–9) (Fig. 1). Among Zambian genotypes one (Coucal) was moderately susceptible while the other two (Loerrie II and Kwale) were susceptible. Of the 55 \( F_2 \) progenies, three were recorded as moderately resistant (score of 4), 48 moderately susceptible (score of 5–6) and four susceptible (score of 7–9) (Fig. 2). No genotype was immune to the disease.

Molecular marker analysis

Marker \( Xgwm570 \) amplified PCR product of 155 base pair (bp) in the moderately resistant parental genotypes 30SAWSN5, 19HRWSN19 and 30SAWSN8 (Table 3). The 155 bp fragment was also present in 25 moderately resistant \( F_2 \) progenies from these parental genotypes, which was absent in the susceptible genotypes. A susceptible parental genotype SB50 (Sonalika) and some \( F_2 \) progenies with SB50 segments showed a 172 bp fragment upon amplification with marker \( Xgwm570 \). The 172 bp amplicon was present in all susceptible genotypes and not in the resistant and moderately resistant genotypes.

There was no amplification with marker \( Xgwm544 \) for moderately resistant parental genotypes 19HRWSN6 and 30SAWSN5. However, 22 \( F_2 \) progenies derived from these parental genotypes, 30SAWSN5 and 19HRWSN6, showed band sizes of 196, 198 and 200 bp (Table 3). Parents 30SAWSN10 and 30SAWSN18 showed a similar band to that observed in the \( F_2 \) progenies. Upon screening with marker \( Xgwm544 \), a 192 bp fragment was observed in the susceptible parents, which included Kwale and Loerrie II and some \( F_2 \) progenies derived from these parents.

Marker \( Xgwm437 \) amplified a 121 bp fragment in the susceptible parent SB50 and \( F_2 \) progenies with fragments of SB50. Marker \( Xgwm437 \) amplified an identical fragment size of 129 and 138 bp (Table 3) in the resistant parental genotype 30SAWSN5 and 19HRWSN6, and 11 \( F_2 \) progenies derived from them.

Single marker regression analysis showed a significant association \((P<0.01)\) between phenotype trait and associated genotyped results for marker \( Xgwm570 \) (Table 4). Marker \( Xgwm570 \) accounted for 14.0\% of phenotypic variation \((R^2)\). A significant association was also observed between genotype results of molecular marker \( Xgwm544 \) \((P<0.05)\) and the associated phenotypic trait. The \( R^2 \) for marker \( Xgwm544 \) was 13.0\%. The association between genotype results of molecular marker \( Xgwm437 \) and the phenotype was significant \((P<0.05)\). The phenotypic variance explained \((R^2)\) by \( Xgwm437 \) was 9.5\%.

The multiple regression model with all the three markers showed a significant association \((P<0.01)\)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Chromosome</th>
<th>Primer sequence (forward and reverse)</th>
<th>Fragment size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Xgwm570 )</td>
<td>6A</td>
<td>5′ TCGGCTTTTACAGTCGGC 3′ 5′ ATGGGTAGCTGAGAGCCAAA 3′</td>
<td>149 143</td>
<td>Röder et al. (1998)</td>
</tr>
<tr>
<td>( Xgwm544 )</td>
<td>5B</td>
<td>5′ TAGAATTCCTATGGGTCTGC 3′ 5′ AGGATTCCAGGCTTCCAAAATT 3′</td>
<td>197 117</td>
<td>Röder et al. (1998)</td>
</tr>
<tr>
<td>( Xgwm437 )</td>
<td>7D</td>
<td>5′ GATCAAGACCTTTTGATCTCCTC 3′ 5′ GATGTTCCAGGCTTCTA 3′</td>
<td>109 111</td>
<td>Röder et al. (1998)</td>
</tr>
</tbody>
</table>

Table 1. Simple sequence repeat markers linked with resistance to Bipolaris sorokiniana, chromosome location and fragment sizes of Opata and Synthetic wheat
with the phenotypic trait (Table 5). The percentage of phenotypic variation ($R^2$) explained by all three markers was 18.0%. Marker Xgwm570 contributed more significantly ($P<0.001$) to the multiple regression model (Table 4) than molecular markers Xgwm544 and Xgwm437.

**DISCUSSION**

Significant differences in the phenotypic data implied that the genotypes differed with respect to their resistance to *B. sorokiniana* at the genotypic level, and hence the population was ideal to use for marker validation.

The results indicated that all the three SSR markers Xgwm570, Xgwm544 and Xgwm437 showed an association with resistance to *B. sorokiniana*, which causes spot blotch disease. Marker Xgwm570 differentiated between resistant and susceptible genotypes by the amplicon size of 155 bp, which amplified only in the resistant and moderately resistant parental genotypes and their derived F$_2$ progenies but not in the susceptible genotypes. The similarity observed in the amplified fragments in the resistant parental and the F$_2$ progenies, resistant and moderately resistant ones, is one of the indications that the marker was associated with resistance (Reena & Jaiwal 2014; Sehrawat et al. 2016). However, all the susceptible parental genotypes and their derived F$_2$ population showed a 172 bp amplification product, confirming the absence of the gene for *B. sorokiniana* resistance. Similarly to the present study, Aggarwal et al. (2011) using a sequence characterized amplified regions (SCAR) marker (SCAR$_{600}$) amplified a 600 bp band in all leaves infected with *B. sorokiniana* but not in healthy leaves. The significance of the regression coefficient ($P<0.01$) showed that marker Xgwm570 was highly associated with resistance to *B. sorokiniana*. This shows that marker Xgwm570 has a high potential for use in MAS for resistance to *B. sorokiniana*. These results confirm earlier findings

<table>
<thead>
<tr>
<th>Source</th>
<th>Degree of freedom</th>
<th>Mean square</th>
<th>Probability value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment (Env)</td>
<td>2</td>
<td>27.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Replication (Env)</td>
<td>3</td>
<td>1.38</td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>65</td>
<td>1.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Genotype × Env</td>
<td>130</td>
<td>0.41</td>
<td>NS</td>
</tr>
<tr>
<td>Error</td>
<td>195</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>395</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS, not significant.

**Table 2. Analysis of variance of spot blotch disease reaction evaluated in three locations in the 2014/15 cropping season**

**Fig. 1. Reaction of parental genotypes to spot blotch disease evaluated in three locations in the 2013/14 cropping season.**

**Table 3. Simple sequence repeat marker, detected alleles, amplified fragment size range (base pair (bp)) and amplification fragment size (bp) in resistant genotypes**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Number of detected alleles</th>
<th>Fragment size range (bp)</th>
<th>Resistant fragment size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xgwm570</td>
<td>6</td>
<td>132–172</td>
<td>155</td>
</tr>
<tr>
<td>Xgwm544</td>
<td>9</td>
<td>177–250</td>
<td>196, 198, 200</td>
</tr>
<tr>
<td>Xgwm437</td>
<td>14</td>
<td>116–157</td>
<td>129, 138</td>
</tr>
</tbody>
</table>

**Fig. 2. Reaction of progenies to spot blotch disease in three locations in the 2013/14 cropping season.**

https://doi.org/10.1017/S0021859617000144
Table 4. Single marker and multiple regression accumulated analysis for resistance to Bipolaris sorokiniana in wheat

<table>
<thead>
<tr>
<th>Single marker analysis</th>
<th>Multiple regression accumulated analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marker</td>
<td>Probability value</td>
</tr>
<tr>
<td>Xgwm570</td>
<td>0.003</td>
</tr>
<tr>
<td>Xgwm437</td>
<td>0.03</td>
</tr>
<tr>
<td>Xgwm454</td>
<td>0.03</td>
</tr>
</tbody>
</table>

NS, not significant

by Sharma et al. (2007), who suggested that the Xgwm570 was linked to resistance genes of B. sorokiniana that cause spot blotch disease.

Marker Xgwm544 also discriminated effectively between susceptible and resistant genotypes. Marker Xgwm544 amplified a 192 bp fragment in susceptible parental genotypes and their derived F2 progenies but not in the resistant genotypes, an indication that the gene for resistance to B. sorokiniana was absent in them. Marker Xgwm544 could not produce any amplicons in the moderately resistant parental genotypes 30SAWSN5 and 19HRWSN6. This could be due to the absence of tested linked SSR polymorphism in these genotypes (Mondal et al. 2008, 2012) or due to crossing over between the marker and the allele for resistance in these parental genotypes (Mondal et al. 2008). Additionally, it could be due to the marker not being tightly linked to the resistant allele in the corresponding genotypes (Gajjar et al. 2014) and/or other genetic factors conditioning resistance (Young & Kelly 1997).

On the other hand, F2 progenies from the resistant parental genotypes (30SAWSN5 and 19HRWSN6) and, 30SAWSN10 and 30SAWSN18 upon screening with Xgwm544 produced 196, 198 and 200 bp fragments that were highly associated with resistance (P < 0.05) to B. sorokiniana. This implies that the F2 progenies may have resistance genes in common. Additionally, some F2 progenies that were phenotypically identified as moderately susceptible also showed 196, 198 and 200 bp upon amplification with Xgwm544 suggesting that they had some resistant genes. The significance of the regression coefficient observed from the regression analysis (P < 0.05) shows that marker Xgwm544 had a relationship with resistance to B. sorokiniana. Xgwm544 explained 13% of the observed total phenotypic variation for resistance. This finding confirmed the earlier study by Kumar et al. (2005) that Xgwm544 was linked to B. sorokiniana resistance genes, indicating its usefulness as a tool for identifying resistant genotypes in early breeding generations.

Marker Xgwm437 discriminated resistant from susceptible genotypes in parents and their F2-derived progenies. This was observed from the products of amplification that were identical in the parental and the F2 progenies. That is, marker Xgwm437 amplified a 121 bp fragment that was only present in the susceptible genotypes, thus, suggesting the absence of resistance genes to B. sorokiniana. Aggarwal et al. (2010) using universal rice primer (URP)-2F observed three bands of 600, 800 and 900 bp only in wheat leaves infected with B. sorokiniana and not in healthy leaves. Marker Xgwm437 amplified identical fragment of 129 bp and 138 in resistant parental genotypes (30SAWSN5 and 19HRWSN6) and F2 moderately resistant progenies confirming the presence of resistance genes to B. sorokiniana. Marker Xgwm437 also displayed a relationship with resistance to B. sorokiniana considering the significance of the regression coefficient (P < 0.05) (Anandhan et al. 2010). Thus, marker Xgwm437 previously reported by Kumar et al. (2005) to be linked with resistance to B. sorokiniana, was also confirmed in the present study. These results mean that marker Xgwm437 will be useful in screening for resistant genotypes as it would accelerate the identification of resistant genotypes during early generations (Bernardo et al. 2013). Multiple regression analysis showed that the addition of markers explained more of the phenotypic variation as observed from the R² value. A significant association between the markers and the phenotypic trait implies that there was a positive interaction effect between the markers (Haley & Knott 1992). Hence, therefore, a combination of the markers can be employed during marker assisted breeding to accelerate identification of resistant genotypes.

Table 5. Multiple regression analysis of resistance to Bipolaris sorokiniana in wheat using three molecular markers, Xgwm570, Xgwm544 and Xgwm437

<table>
<thead>
<tr>
<th>Source</th>
<th>Degree of freedom</th>
<th>Mean square</th>
<th>Probability value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>3</td>
<td>2.63</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Residual</td>
<td>63</td>
<td>23.94</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>0.58</td>
<td></td>
</tr>
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</table>

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CONCLUSION

The SSR markers Xgwm570, Xgwm544 and Xgwm437 previously reported to be linked with resistance to *B. sorokiniana*, which causes spot blotch disease in wheat, were validated in the present study. The results suggest that the markers could be useful in MAS in increasing the efficiency for identification of resistant genotypes in the seedling stage even in the absence of the disease epiphytotic conditions. Since the $R^2$ values are low, a combination of two or three SSR markers can be employed during MAS. This was evident by the multiple linear equation which gave a combined $R^2$ value of 18-0%, obtained from inclusion of all the three markers.

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