Marker-assisted selection for sheath blight resistance in rice (Oryza sativa L.)

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Abstract
Sheath blight is one of destructive diseases of rice, causing substantial loss to rice production. Developing resistant cultivars to sheath blight is quite difficult due to lack of effective resistant source in the available germplasm. Pyramiding of genes/QTLs for different biotic stresses gives broad-spectrum resistance to multiple diseases for any crop. The present study was carried out to introgress sheath blight resistant QTL, qSBR11-1, into the backgrounds of CB14004 and CB14002 from the donor parent Tetep. CB14004 and CB14002 are the two bacterial blight resistant genotypes, pyramided with three bacterial blight resistant genes viz., xa5, xa13 and Xa21. The positive plants of BC3F1 individuals for sheath blight QTL i.e., qSBR11-1 were self-pollinated to generate BC3F2 individuals. The BC3F2 individuals were screened with linked molecular marker of qSBR11-1 i.e., RM 224 and the confirmed plants were assessed for morphological traits to identify the superior segregates.

Keywords
Rice, marker-assisted selection, sheath blight resistance

INTRODUCTION
Rice (Oryza sativa L.) is one of the major staple food crops for more than 50% of world population (Sharma et al., 2012). The population may increase to 9 billion by the end of 2050 and food production is sufficient to meet the requirements of only 60% of the population (FAO, 2018). China is the leading producer of rice (142.3 million tonnes) followed by India (110.4 million tonnes) (FAO, 2018), thereby plays a major role in meeting the rice demand. In India, rice shared by 48% of total food grain production and it is main source of income to many people for meeting their daily requirements (Kiruthikadevi et al., 2020). Rice production is affected by several pathogens, which are major threats for food security. This threat can be addressed and production can be enhanced by introgressing the resistant genes/QTLs, which are available in the wild species and germplasm accessions into cultivated varieties. Recent advances in biotechnology, enhances the application of molecular markers for precise introgression of resistant genes/QTLs into the targeted varieties. Many varieties and improved lines have been developed for the benefit of farmers for different stresses through marker-assisted selection (Sundaram et al., 2008; Ramalingam et al., 2017; Chithramneenal et al., 2018).

Rice sheath bight, caused by Rhizoctonia solani Khun, is considered as one of the important diseases of rice and it causes a yield loss up to 45% (Margani et al., 2018). Sheath blight is the second most important disease of rice next to blast (Susmita Dey et al., 2020). R. solani is a necrotic soil borne pathogen, survives in the crop residues of rice as sclerotia or in the form of mycelium. The sclerotia of the infected plant floats on the water surface and germinates on the rice sheath, forms appressorium (Richa et al., 2016). The appressorium causes the initial infection by colonizing the entire plant through the surface hyphae and causes necrotic damage in the sheath region (Ou, 1985). The disease aggressive at the time of panicle differentiation stage and inhibits grain filling. Till date, none of the varieties having resistance to ShB were...
Marker-assisted selection for sheath blight

reported (Channamallikarjuna et al., 2010). Sheath blight is challenging to control because of its wide host ranging capacity and persistence of sclerotia in higher climatic conditions (Pooja Singh et al., 2019). Many QTLs have been identified in the background of Tetered linked to molecular markers through QTL mapping approach. Among them, qSBR11-1 (linked to RM 224), providing a moderate resistance to ShB across the years and locations (Channamallikarjuna et al., 2010). In the present study, qSBR11-1 was introgressed in the backgrounds of CB14004 and CB14002 for moderate resistance to sheath blight disease.

MATERIALS AND METHODS

CB14004 and CB14002 are the two bacterial blight resistant genotypes, pyramided with xa5, xa13 and Xa27, developed at TNAU, Coimbatore (Perumalsamy et al., 2009). Both CB14004 and CB14002 were used as recurrent parents for introgression of sheath blight resistance. Tetered, a Vietnamese indica landrace conferring moderately resistance to sheath blight (Channamallikarjuna et al., 2010) was used as donor parent for targeted introgression of sheath blight resistant QTL i.e., qSBR11-1 into the backgrounds of CB14004 and CB14002 to combine sheath blight resistance with bacterial blight resistance in the recurrent parents.

BCF1 and BCF2 hybrids have been developed and fixed for sheath blight resistance in the backgrounds of CB14004 and CB14002 (Vidya et al., 2018). BCF1 hybrids were developed by crossing CB14004 × Tetered and CB14002 × Tetered to combine both sheath blight and bacterial blight resistance in the backgrounds of CB14004 and CB14002. RM 224 is a closely linked molecular marker of qSBR11-1, explains 14% of phenotypic variation and mapped on the chromosome number 11 (Channamallikarjuna et al., 2010). The BCF1 hybrids were screened with linked molecular marker RM 224 to confirm the targeted QTL in heterozygous condition. The plants showing heterozygous for the targeted QTL were self-pollinated to generate BCF2 individuals. The BCF2 segregates were screened with linked molecular marker of qSBR11-1 to fix the plants for sheath blight resistance. The confirmed plants for sheath blight resistance were also assessed for morphological and grain quality traits, to select the segregates similar to recurrent parents.

DNA was extracted from the fresh young leaves of BCF1 and BCF2 individuals along with parents at maximum tillering stage by CTAB method (Sambrook et al., 1989). DNA quality was checked by 0.8% agarose gel followed by quantified with spectrophotometer and the concentration of DNA was adjusted to 50ng/μl. 10 μl of PCR reaction mixture contains 4 μl of smart prime 2X master mix, 4 μl of water, 1 μl of template DNA, 0.5 μl of each forward and reverse primers. PCR was conducted in the Effendorf thermocycler with following protocol: initial denaturation of 94°C for 4 min, followed by 35 cycles of denaturation of 94°C for 1 min, annealing at 55°C for 1 min, initial extension of 72°C for 1 min and final extension of 72°C for 7 min. The PCR products were separated by using 3% agarose gel stained with ethidium bromide and visualized on UV light.

RESULTS AND DISCUSSIONS

The present study was carried out to introgress sheath blight resistance into the backgrounds of popular varieties (CB14004 and CB14002) harboring BB resistance genes (xa5, xa13 and Xa27) through marker-assisted selection. A total of 143 BCF2 hybrids were produced by crossing with the positive plants of BCF1 hybrids with recurrent parents (CB14004 and CB14002).

Table 1. Agro-morphological characters of selected lines harboring ShB QTL

<table>
<thead>
<tr>
<th>Genotype</th>
<th>PH (cm)</th>
<th>DFF</th>
<th>NPT</th>
<th>PL (cm)</th>
<th>NGP</th>
<th>SPY (g)</th>
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<tbody>
<tr>
<td>IL # 1-1-3</td>
<td>85.3</td>
<td>82</td>
<td>17</td>
<td>23.2</td>
<td>197</td>
<td>25.6</td>
</tr>
<tr>
<td>IL # 1-1-9</td>
<td>79.4</td>
<td>87</td>
<td>18</td>
<td>25.5</td>
<td>190</td>
<td>26.5</td>
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<tr>
<td>IL # 1-2-5</td>
<td>88.8</td>
<td>80</td>
<td>16</td>
<td>22.5</td>
<td>188</td>
<td>24.3</td>
</tr>
<tr>
<td>IL # 1-2-8</td>
<td>79.2</td>
<td>88</td>
<td>19</td>
<td>25.1</td>
<td>203</td>
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<tr>
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<td>81</td>
<td>16</td>
<td>22.8</td>
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<td>24.1</td>
</tr>
<tr>
<td>IL # 1-5-4</td>
<td>83.1</td>
<td>81</td>
<td>15</td>
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<td>IL # 2-1-9</td>
<td>87.9</td>
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<td>16</td>
<td>25.6</td>
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<td>16</td>
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<tr>
<td>IL #2-6-8</td>
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<td>79</td>
<td>17</td>
<td>24.2</td>
<td>190</td>
<td>25.6</td>
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<td>CB14002</td>
<td>84.5</td>
<td>85</td>
<td>16</td>
<td>22.5</td>
<td>191</td>
<td>26.1</td>
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<td>CB14004</td>
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<td>84</td>
<td>17</td>
<td>23.9</td>
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<td>27</td>
</tr>
<tr>
<td>Tetered</td>
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<td>91</td>
<td>20</td>
<td>27.2</td>
<td>155</td>
<td>29.4</td>
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<tr>
<td>Mean</td>
<td>86.91</td>
<td>81.71</td>
<td>17</td>
<td>23.82</td>
<td>188.35</td>
<td>25.57</td>
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<td>SD</td>
<td>10.38</td>
<td>4.65</td>
<td>1.26</td>
<td>1.26</td>
<td>10.99</td>
<td>1.44</td>
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<td>CV (%)</td>
<td>11.95</td>
<td>5.69</td>
<td>7.44</td>
<td>5.30</td>
<td>5.83</td>
<td>5.64</td>
</tr>
</tbody>
</table>

PH - Plant height (cm); DFF - Days to 50% flowering; NPT - Number of productive tillers; PL - Panicle length (cm); NGP - Number of grains per panicle; SPY - Single plant yield (g)

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All the 143 BC$_3$F$_1$ hybrids were sown and analyzed with linked molecular marker of qSBR11-1 i.e., RM 224. Foreground analysis reveals four plants in CB14004 × Tetep combination and seven plants in CB14002 × Tetep combination were showing heterozygous for the targeted QTL i.e., qSBR11-1. The hybrids selected by genotypic screening method were self-pollinated to generate BC$_3$F$_2$ individuals. A total of 400 plants in the both combinations (CB14004 × Tetep and CB14002 × Tetep) were first screened linked molecular marker and the positive plants were assessed phenotypically to select the plants having superior agronomic characters. By screening genotypic and phenotypic screening methods, we have found 25 lines in CB14004 background and 21 lines in CB14002 background having presence of targeted sheath blight resistant QTL(qSBR11-1). Further, the segregates possessing sheath blight resistance were also assessed for key morphological traits viz., plant height (cm), the number of productive tillers, days to 50% flowering, panicle length (cm), the number of grains per panicle, and single plant yield (gm) to identify the lines similar to recurrent parents. The evaluation of morphological traits reveals, most of the selected lines harbouring qSBR11-1 were have similar morphological traits as recurrent parents (Table.1). This indicates, the identified segregants were a result of combination of marker-assisted selection and phenotypic selection to have the recurrent parent alleles and donor parent alleles. Channamallikarjuna et al., (2010) have identified qSBR11-1, on the chromosome number 11 through composite interval mapping. Among the 12 QTLs identified in the recombinant lines of HP2216 (susceptible) and Tetep (resistant), qSBR11-1 explains 14% of phenotypic variation. The candidate QTL, qSBR11-1 was also found consistently over the four years and two locations. The present study was focuses on the introgression of qSBR11-1 QTL for moderate resistance to sheath blight in rice. Pyramiding of two or three major genes/QTLs enhances the resistance levels for different isolates of pathogens than a single gene resistance (Sundaram et al., 2008). Introggression of sheath blight resistance in the background of cultivars harbouring bacterial blight resistance enhances the resistance levels to two different biotic stresses. Singh et al., (2012) first time introgressed qSBR11-1, in the background of improved Pusa Basmati, to combine the sheath blight resistance along with bacterial blight resistance. The results of Singh et al., (2012) were in accordance with the present study results. Further, the lines harbouring ShB resistant QTL need to assessed for physical resistance against the isolates of rice sheath blight pathogen at BC$_3$F$_3$ generation. The present study demonstrates precise introgression of targeted QTL for sheath blight resistance in rice.

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