Research Application Summary

Enhancing soybean rust resistance through gene pyramiding

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Abstract

The threat posed by soybean rust (Phakopsora pachyrhizi) on soybean production is worsened by resistance breakdown associated with single gene resistance present in most cultivars. Few studies have, however, been undertaken to use mapped simple sequence markers for gene pyramiding to enhance rust resistance. This study validated use of identified simple sequence repeat markers for gene pyramiding and determined the most effective pairwise gene combination for three independent soybean rust resistance genes, Rpp2, Rpp3 and Rpp4. In the F2 generation, soybean plants (homozygous dominant or heterozygous at both loci) with two gene combinations had relatively lower disease severity and sporulation than the parents suggesting complementary epistatic gene action for resistance. Similarly, homozygous F1 families showed lower severity, lesion density and sporulation. Gene Rpp3 contributed positively to resistance with various genetic backgrounds for most parameters measured compared to Rpp2 and Rpp4 resistance genes. Overall, the results suggest that marker gene pyramiding is feasible and can substantially increase resistance to soybean rust through reduced severity and reduced sporulating lesions.

Key words: Epistasis, genetic background, Phakopsora pachyrhizi, simple sequence repeats, soybeans

Résumé

La menace posée par la rouille du soja (Phakopsora pachyrhizi) sur la production du soja est aggravée par la dégradation de la résistance associée à une simple résistance génique présente dans la plupart de cultivars. Peu d’études ont toutefois été menées pour utiliser des marqueurs séquentiels simples cartographiés pour la pyramidation génique afin d’améliorer la résistance à la rouille. Cette étude a validé l’utilisation de simples marqueurs derépétition séquentielleidentifiés pour la pyramidation génique et a déterminé la combinaison génique efficace des paires pour trois gènes indépendants de résistance à la rouille du soja, Rpp2, Rpp3 et Rpp4. Dans la génération de F2, les plants du soja (homozygotes ou hétérozygotes dominant aux deux locus) avec
Background

Gene pyramiding, which involves assembling multiple desirable genes into a single genotype has been suggested as a method that can overcome resistance instability conferred by single gene resistance to soybean rust, *Phakopsora pachyrhizi* (Hartman *et al.*, 2005; Garcia *et al.*, 2008; Yamanaka *et al.*, 2010; Lemos *et al.*, 2011). Our aim for pyramiding rust resistance genes in this study was to enhance soybean rust resistance to field isolates and broaden the genetic base for rust resistance in the available soybean breeding lines. However, incorporating such multiple gene resistance has remained a challenge using conventional methods due to the requirement of extensive screening using gene specific pathogen races (Sanghai-Maroof *et al.*, 2008). Conventional approaches are not always practically feasible in gene pyramiding given the fact that some genes were identified using foreign races of soybean rust whose access presents logistical and phyto-sanitary challenges. Accordingly, marker assisted selection was the most desirable alternative available for pyramiding resistance genes. Therefore the objectives of this study were to validate the use of marker assisted selection in F$_2$ and F$_2$:3, families to pyramid three resistance genes in pairwise combinations and determine the most effective gene combinations for enhancing resistance to soybean rust.

In soybean, resistance to rust is manifested phenotypically by red brown lesions (Bromfield, 1984; Bonde *et al.*, 2006), and is conditioned by six major resistance genes R$_{pp1}$, R$_{pp2}$, R$_{pp3}$, R$_{pp5}$, R$_{pp}$, R$_{pp}$, which have been mapped to different linkage groups. R$_{pp1}$ linkage group (LG) G (Hyten *et al.*, 2007), R$_{pp3}$ LGC2 (Hyten *et al.*, 2009), R$_{pp2}$ and R$_{pp4}$
Gene pyramiding was done through single crosses in a screen house using parental lines: PI 230970, Ankur and PI 459025 having three specific resistance genes R_{pp2}, R_{pp3} and R_{pp4} respectively. The crosses were done in pair wise combinations at Makerere University Agricultural Research Institute (MUARIK) during 2009 season and were implemented as follows; PI 230970 (R_{pp2}) X Ankur (R_{pp3}); PI 230970 (R_{pp2}) X PI 459025 (R_{pp4}) and Ankur (R_{pp3}) X PI 459025 (R_{pp4}). The crossing scheme and progeny selection is presented in Figure 1.

Hybrids from R_{pp2} x R_{pp3}, R_{pp2} x R_{pp4}, R_{pp3} x R_{pp4} gene combinations were assessed for disease severity

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**Figure 1.** Gene pyramiding scheme used for resistance genes R_{pp2}, R_{pp3} and R_{pp4}, and the generations where marker assisted selection was done. ☉ represents self pollination.
compared to parental lines with single genes starting from the 
R5 stage (Fehr et al., 1971). Rust severity was determined at 
weekly intervals, using a scale based on the counted lesion 
density per leaflet, where 1 = no lesions; 2 = 1-30; 3 = 31-75; 4 = 76-
150; 5 = 151-300; 6 = 301-750; 7 = 751-500; 8 = 1501-3000 and 
9 = >3000 lesions from three trifoliates of the mid-canopy (Miles 
et al., 2008). Sporulation rate was evaluated based on a 1-to-5 
scale (where 1 represents no sporulation and 5 profuse 
sporulation). Using x20 magnification lenses soybean lines were 
evaluated for the number of lesions per square centimetre, 
proportion of uredinia with lesions. Numbers of pustules per 
lesion were also assessed after vacuuming selected leaves with 
a hand held Liliput® vacuum to dislodge any urediniospores for 
easy counting. Parents were assayed for polymorphism using 
the six SSR primers prior to F2 and F2:3 progeny screening.

Table 1. Severity and sporulation rate of genotyped F2 plants evaluated at two time intervals.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of plants evaluated</th>
<th>Severity</th>
<th>Sporulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>Parents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rpp2 Rpp2</td>
<td>98</td>
<td>3.66±0.26</td>
<td>3.66±0.26</td>
</tr>
<tr>
<td>Rpp3 Rpp3</td>
<td>98</td>
<td>3.33±0.28</td>
<td>4.33±0.25</td>
</tr>
<tr>
<td>Rpp4 Rpp4</td>
<td>98</td>
<td>4.00±0.35</td>
<td>4.40±0.26</td>
</tr>
<tr>
<td>F2 plants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rpp2 X Rpp3</td>
<td>27</td>
<td>2.88±0.46</td>
<td>3.38±0.28</td>
</tr>
<tr>
<td>Rpp3 X Rpp4</td>
<td>19</td>
<td>2.66±0.35</td>
<td>3.16±0.23</td>
</tr>
<tr>
<td>Rpp2 X Rpp4</td>
<td>11</td>
<td>2.40±0.50</td>
<td>2.60±0.34</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>3.18±0.13</td>
<td>3.59±0.12</td>
</tr>
</tbody>
</table>

T-Time; at R5 and after one week later, ± standard error; notation Rpp_ implies the alternative allele was either dominant or recessive.
The family derived from \textit{Rpp2Rpp2} \textit{Rpp3Rpp3} had the least lesions per square centimetre and frequency of lesions with uredinia (Table 2). The family derived from \textit{Rpp2Rpp2} \textit{Rpp4Rpp4} had a severity score lower than all the parents evaluated. However, its sporulation rate was higher than parents \textit{Rpp3Rpp3} and \textit{Rpp4Rpp4}. The numbers of pustules per lesion were not significantly different for all the genotypes evaluated. Though the presence of multiple virulence in soybean rust was seen as the main challenge to the efficacy of gene pyramiding (Shanmugasundaram \textit{et al.}, 2004), our study noted increased resistance in the two gene combinations. Furthermore, our results suggest that the utilisation of marker assisted selection in pyramiding soybean rust resistance genes is possible. Although the number of lines tested was small, the results from our study clearly demonstrate that pyramiding \textit{Rpp2Rpp2} \textit{Rpp3Rpp3} in homozygous condition increases resistance. All the genes tested contributed additively to resistance, though, in a disproportionate manner. Introgession of these double resistance gene genotypes into farmer preferred cultivars is therefore recommended. This study did not focus on the durability aspect of the resistance genes which is crucial for any resistance breeding programme. Consequently, further research on evaluating soybean resistance genes for durability

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Disease severity</th>
<th>Lesions/cm²</th>
<th>Reaction</th>
<th>% Uredinia type with sporulating lesions</th>
<th>Pustules per lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{Rpp2Rpp2}</td>
<td>4.91±0.48</td>
<td>45.39±5.68</td>
<td>RB</td>
<td>100</td>
<td>2.0</td>
</tr>
<tr>
<td>\textit{Rpp3Rpp3}</td>
<td>3.28±0.39</td>
<td>24.52±4.56</td>
<td>RB</td>
<td>18</td>
<td>1.5</td>
</tr>
<tr>
<td>\textit{Rpp4Rpp4}</td>
<td>3.20±0.33</td>
<td>24.93±3.90</td>
<td>RB</td>
<td>38</td>
<td>1.1</td>
</tr>
<tr>
<td>\textit{F\textsubscript{2:3}} families</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{Rpp2Rpp2} X \textit{Rpp3Rpp3}</td>
<td>2.62±0.48</td>
<td>18.41±3.16</td>
<td>RB</td>
<td>16</td>
<td>0.9</td>
</tr>
<tr>
<td>\textit{Rpp2Rpp2} X \textit{Rpp4Rpp4}</td>
<td>3.02±0.30</td>
<td>26.21±3.49</td>
<td>RB</td>
<td>15</td>
<td>1.9</td>
</tr>
<tr>
<td>Mean</td>
<td>3.14±0.19</td>
<td>24.95±2.62</td>
<td></td>
<td>24</td>
<td>1.2</td>
</tr>
<tr>
<td>\textit{F prob}</td>
<td>\textless0.05</td>
<td>\textless0.05</td>
<td>\textless0.01</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

\textit{± standard error; ns- non-significant; <0.05; <0.01; <0.001}

Table 2. Disease response parameters for the parents and ten homozygous dominant plants from \textit{F\textsubscript{2:3}} families.

\textbf{Recommendation}

The presence of multiple virulence in soybean rust was seen as the main challenge to the efficacy of gene pyramiding (Shanmugasundaram \textit{et al.}, 2004), our study noted increased resistance in the two gene combinations. Furthermore, our results suggest that the utilisation of marker assisted selection in pyramiding soybean rust resistance genes is possible. Although the number of lines tested was small, the results from our study clearly demonstrate that pyramiding \textit{Rpp2Rpp2} \textit{Rpp3Rpp3} in homozygous condition increases resistance. All the genes tested contributed additively to resistance, though, in a disproportionate manner. Introgession of these double resistance gene genotypes into farmer preferred cultivars is therefore recommended. This study did not focus on the durability aspect of the resistance genes which is crucial for any resistance breeding programme. Consequently, further research on evaluating soybean resistance genes for durability.
Acknowledgement

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References


