

## 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 F<sub>2</sub> 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 F<sub>3</sub> 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 de répétition séquentielle identifiés 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 F<sub>2</sub>, les plants du soja (homozygotes ou hétérozygotes dominant aux deux locus) avec

deux combinaisons de gènes avaient une sévérité et une sporulation de la maladie relativement plus faibles que les parents suggérant une action complémentaire génique épistatique pour la résistance. De même, les familles homozygotes de F<sub>3</sub> ont montré une sévérité plus faible, une densité de la lésion et une sporulation. Le gène *Rpp3a* contribué positivement à la résistance à divers patrimoines génétiques pour la plupart des paramètres mesurés par rapport aux gènes de résistance de *Rpp2* et *Rpp4*. Dans l'ensemble, les résultats suggèrent que la pyramidation des gènes du marqueur est faisable et peut augmenter considérablement la résistance à la rouille du soja par la sévérité réduite et les lésions de la sporulation réduites.

Mots clés: épistasie, patrimoine génétique, *Phakopsora pachyrhizi*, répétitions séquentielles simples, soja

## 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-Maroo 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<sub>2</sub> and F<sub>2,3</sub> families to pyramid three resistance genes in pair-wise combinations and determine the most effective gene combinations for enhancing resistance to soybean rust.

## Literature Summary

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 *Rpp1*, *Rpp2*, *Rpp3*, *Rpp3*, *Rpp4*, *Rpp5*, *Rpp<sub>Hyyuga</sub>* which have been mapped to different linkage groups. *Rpp1* linkage group (LG) G (Hyten et al., 2007), *Rpp3* LGC2 (Hyten et al., 2009), *Rpp2* and *Rpp4*

LGJ and G respectively (Silva *et al.*, 2008; Yamanaka *et al.*, 2008), *Hyuuga* LGC2 (Monteros *et al.*, 2007) and *Rpp5* LGN (Garcia *et al.*, 2008). Long term utilisation of these race specific resistance genes has prompted the pathogen to mutate and overcome them. Empirical evidence in Africa, Orient and South America has shown that some of the once effective soybean rust resistance genes have been overcome by new rust races (Tschanz *et al.*, 1986; Hartman *et al.*, 2004; Laperuta *et al.*, 2008). Despite resistance breakdown associated with race specific genes, they are still effective against a broad range of pathotypes in wheat rust pathosystems (Pfender, 2009). The relative ease with which such monogenic resistance is detected and introgressed into susceptible backgrounds makes it a high potential rapid mitigation strategy against soybean rust.

### Study Description

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 *Rpp2*, *Rpp3* and *Rpp4* 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 (*Rpp2*) X Ankur (*Rpp3*); PI 230970 (*Rpp2*) X PI 459025 (*Rpp4*) and Ankur (*Rpp3*) X PI 459025 (*Rpp4*). The crossing scheme and progeny selection is presented in Figure 1.

Hybrids from *Rpp2* x *Rpp3*, *Rpp2* x *Rpp4*, *Rpp3* x *Rpp4* gene combinations were assessed for disease severity

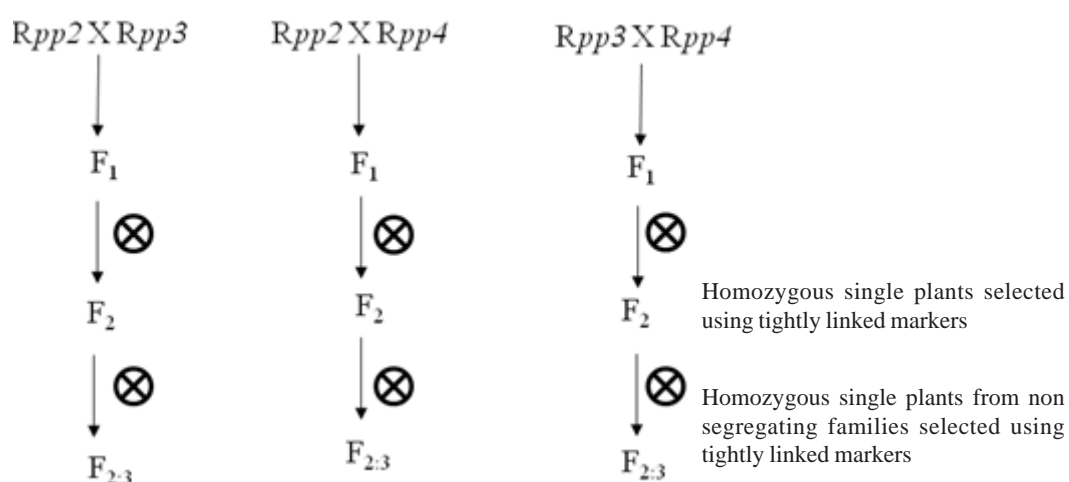


Figure 1. Gene pyramiding scheme used for resistance genes *Rpp2*, *Rpp3* and *Rpp4*, and the generations where marker assisted selection was done.  $\otimes$  represents self pollination.

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 F<sub>2</sub> and F<sub>2,3</sub> progeny screening.

### Research Application

The parental lines with the *Rpp2* gene remained unchanged in severity during the two time intervals of disease evaluation. All parents had higher severity compared to the selected plants with two gene combinations. The cross *Rpp3*\_x *Rpp4*\_ had a significantly lower severity followed by *Rpp2*\_x *Rpp4*\_ for the two time intervals. Contrary to other genotypes, sporulation decreased from 3.3 to 2.4 for parent with the gene *Rpp2*. Progeny from *Rpp2*\_x *Rpp4*\_ similarly had low sporulation rate followed by *Rpp2*\_x *Rpp3*\_.

**Table 1. Severity and sporulation rate of genotyped F<sub>2</sub> plants evaluated at two time intervals.**

Genotype	No. of plants evaluated	Severity		Sporulation	
		T1	T2	T1	T2
<b>Parents</b>					
<i>Rpp2</i> <i>Rpp2</i>	98	3.66±0.26	3.66±0.26	3.33±0.27	2.44±0.34
<i>Rpp3</i> <i>Rpp3</i>	98	3.33±0.28	4.33±0.25	3.16±0.23	3.16±0.23
<i>Rpp4</i> <i>Rpp4</i>	98	4.00±0.35	4.40±0.26	1.40±0.75	3.20±0.61
<b>F<sub>2</sub> plants</b>					
<i>Rpp2</i> _ X <i>Rpp3</i> _	27	2.88±0.46	3.38±0.28	2.00±0.61	2.44±0.34
<i>Rpp3</i> _ X <i>Rpp4</i> _	19	2.66±0.35	3.16±0.23	2.33±0.34	2.83±0.24
<i>Rpp2</i> _ X <i>Rpp4</i> _	11	2.40±0.50	2.60±0.34	1.40±0.75	2.40±0.58
Mean		3.18±0.13	3.59±0.12	2.60±0.15	2.62±0.13

T-Time; at R5 and after one week later, ± standard error; notation *Rpp*\_ implies the alternative allele was either dominant or recessive.

**Table 2. Disease response parameters for the parents and ten homozygous dominant plants from F<sub>2:3</sub> families.**

Genotype	Disease severity	Lesions/cm <sup>2</sup>	Reaction	% Uredinia type with sporulating lesions	Pustules per lesion
<b>Parents</b>					
<i>Rpp2Rpp2</i>	4.91±0.48	45.39±5.68	RB	100	2.0
<i>Rpp3Rpp3</i>	3.28±0.39	24.52±4.56	RB	18	1.5
<i>Rpp4Rpp4</i>	3.20±0.33	24.93±3.90	RB	38	1.1
<b>F<sub>2:3</sub> families</b>					
<i>Rpp2Rpp2</i> X <i>Rpp3Rpp3</i>	2.62±0.48	18.41±3.16	RB	16	0.9
<i>Rpp2Rpp2</i> X <i>Rpp4Rpp4</i>	3.02±0.30	26.21±3.49	RB	15	1.9
Mean	3.14±0.19	24.95±2.62		24	1.2
<i>F</i> prob	≤0.05	≤0.05		≤0.01	ns

± standard error; ns- non-significant

The F<sub>2:3</sub> family *Rpp2Rpp2* x *Rpp3Rpp3* had the least lesions per square centimetre and frequency of lesions with uredinia (Table 2). The family derived from *Rpp2Rpp2* x *Rpp4Rpp4* had a severity score lower than all the parents evaluated. However, its sporulation rate was higher than parents *Rpp3Rpp3* and *Rpp4Rpp4*. The numbers of pustules per lesion were not significantly different for all the genotypes evaluated.

## Recommendation

Though the presence of multiple virulence in soybean rust was seen as the main challenge to the efficacy of gene pyramiding (Shanmugasundaram *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 *Rpp2Rpp2* x *Rpp3Rpp3* in homozygous condition increases resistance. All the genes tested contributed additively to resistance, though, in a disproportionate manner. Introgression 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

and using diverse pathogen populations is recommended as this is important for sustainable soybean production.

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