

Pyramiding *Fusarium* root rot resistance genes and validation of SSR PVBR87 in common bean

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Abstract

This study determined effectiveness of pyramided *Fusarium* root rot (FRR) resistance genes and validated association of SSR PVBR87 marker with FRR resistance in common bean. A double cross (DC) involving four resistance sources was used to accumulate FRR resistance genes into one background. The DC F₁ and each resistant line were crossed to two susceptible cultivars. Parents, F₁ and F₂ populations were subjected to FSP-3 in a greenhouse. Two single cross (SC) F₂ populations were screened with SSR PVBR87 marker. Five-parent crosses performed better than single crosses. SSR PVBR87 marker showed association with FRR resistance in the two SC F₂ populations.

Key words: *Fusarium* root rot, FSP-3 (*Fusarium solani* f. sp. *phaseoli* isolate-3), gene pyramiding, SSR (simple sequence repeat)

Résumé

Cette étude a déterminé l'efficacité des gènes pyramidés de résistance de la putréfaction de racine de *Fusarium* (FRR) et a validé l'association du marqueur SSR PVBR87 avec la résistance de FRR dans le haricot ordinaire. Un double croisement (DC) impliquant quatre sources de résistance a été employé pour accumuler des gènes de résistance FRR au second plan. Le F₁ du double croisement DC et chaque lignée de résistante ont été croisés à deux cultivars sensibles. Les parents et les populations F₁ et F₂ ont été soumis à FSP-3 dans un milieu d'observation. Deux populations F₂ de simple croix (SC) ont été examinées avec le marqueur SSR PVBR87. Les croisements de cinq parents se sont exécutés mieux que les croisements simples. Le marqueur SSR PVBR87 a montré l'association avec la résistance FRR dans les deux populations F₂ de SC.

Mots clés: Putréfaction de racine de *Fusarium*, FSP-3 (espèces de *Fusarium solani* f. isolate-3 de *phaseolus*), « pyramidage » de gène, SSR (répétition simple de séquence)

Background

Fusarium root rot (FRR) caused by *Fusarium solani* f. sp. *phaseoli* is among the most serious diseases of common bean in Uganda causing total crop loss in susceptible varieties. Genetic resistance is the most economical and environmentally appropriate strategy for controlling FRR. Breeding progress for FRR resistance has been slow due to the quantitative nature of its genetics. Studies have shown that the number of genes governing resistance to FRR varies from 2-9 among different sources and that the genes are located on different loci. It is proposed that accumulation of these genes into a single genetic background would result in increased level, and more effective transfer of FRR resistance into consumer-preferred varieties. Phenotypic selection for FRR resistance is destructive and strongly influenced by the environment. Use of molecular markers together with phenotypic selection could speed up breeding progress for FRR resistance. Simple sequence repeat (SSR) PVBR87 marker linked to FRR resistance quantitative trait loci (QTL) was identified but its use in selecting resistant genotypes outside its mapping population has not been determined. The objectives of this study were to: determine number of pyramided FRR resistance genes and their interactions in common bean, determine effectiveness of pyramiding resistance genes from different sources in improving FRR resistance levels and validate SSR PVBR87 marker.

Literature Summary

Gene pyramiding is the accumulation of desirable genes into a single line or cultivar (Kloppers and Pretorius, 1997). Application of gene pyramiding for disease resistance in common bean is well documented (Teran and Singh, 2009). Marker validation refers to the process of confirming the effectiveness of markers associated with putative QTL in one population for indicating the target phenotype in independent populations and other germplasm, which incorporate those QTL (Collard *et al.*, 2001). Marker validation is one of the critical steps in developing markers for marker assisted selection (Collard *et al.*, 2005).

Study Description

The study was conducted at the International Centre for Tropical Agriculture (CIAT) based at the National Agricultural Research Laboratories – Kawanda, Uganda. Four FRR resistant (R) inbred lines: MLB-48-89A (48), MLB-49-89A (49), G2333 (G2) and G685 (G6), and two susceptible (S) lines: K20 and Kanyebe (Kan) were used in the study. A double cross was developed from the four resistant parents. DC F₁ and each resistant parent were crossed to the two susceptible cultivars. Parental, F₁ and F₂ populations were subjected to *Fusarium solani* f. sp.

Research Application

phasoeli isolate-3. 21 days after planting symptom severity was assessed on a scale of 1-9. Plants of each cross were grouped into resistant (score 1-4) and susceptible (score 5-9). F₂ plants of K20 x G2 and 49 x Kan were screened with SSR PVBR87 marker. Data were analysed using GenStat and MS-excel computer packages. Number of genes and gene interactions were determined using X² goodness of fit test (P = 0.05). Means were compared by pair wise t-test (P = 0.05). Association of SSR PVBR87 to FRR resistance was determined using X² test of independence and single marker regression analysis (P = 0.05).

Minimum of 2 and 3 genes segregated in the F₂ of R x R single and double crosses, respectively (Table 1). The genes showed dominance, epistatic and recessive gene interactions.

The mean FRR severity in the F₂ of the FPC K20 x [(49x48)x(G2xG6)] was significantly different (P<0.05) from the mean FRR severity in the F₂ of the SC (Table 2). Whereas the F₂ of Kan x [(49x48)x(G2xG6)] was not significantly different (P≥0.05) from F₂ of Kan x 48 and Kan x 49, it performed significantly better (P<0.05) than F₂ of Kan x G2 and Kan x G6 (Table 3). SSR PVBR87 was significantly

Table 1. Chi-square test for goodness of fit of phenotypic classes in F₂.

Genotype	Observed ratio	Hypothesised ratio	Gene number	X ²	Prob
	R : S	R : S			
G2 x G6	232:15	15:1	2	0.01	0.92
49 x 48	233:10	15:1	2	1.77	0.18
(49x48)x(G2xG6)	501:28	61:3	3	0.38	0.54

R = frequency of resistant plants; S = frequency of susceptible plants, X² = chi-square value.

Table 2. Mean F₂ deviations of FPC from SC in K20 crosses.

SC	Mean	FPC _{K20} = 4.38
		FPC - SC
K20 x 48	5.10	-0.72*
K20 x 49	5.22	-0.84*
K20 x G2	5.29	-0.91**
K20 x G6	5.40	-1.02**

Table 3. Mean F₂ deviations of FPC from SC in Kanyebeba crosses.

SC	Mean	FPC _{Kan} = 5.4
		FPC - SC
Kan x 48	6.2	-0.75 ^{ns}
Kan x 49	6.3	-0.85 ^{ns}
Kan x G2	7.0	-1.58***
Kan x G6	6.6	-1.22**

FPC = five-parent cross; SC = single cross; FPC_{K20} = K20x[(49x48)x(G2xG6)]; FPC_{Kan} = Kan x [(49x48)x(G2xG6)]; FPC-SC = FPC deviation from SC; ns = not significantly different from zero at P = 0.05, *, **, *** significantly different from zero at P ≤ 0.05, 0.01, and 0.001, respectively.

associated ($X^2 = 24.7$, $P = 0.0001$, $Df = 2$, $R^2 = 0.13$) with FRR resistance in the K20 x G2. Similarly, the marker showed significant association ($X^2 = 7.3$, $P = 0.03$, $Df = 2$ and $R^2 = 0.04$) with FRR resistance.

Recommendation

Pyramiding FRR resistance genes by combining parental lines with complementary desirable genes followed by selecting desirable recombinants from among the progeny population be used to improve FRR resistance in common bean and (ii) SSR PVBR87 marker could be used together with phenotypic selection to identify FRR resistant bean genotypes but there is need for further testing under different environmental conditions.

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