Research Application Summary

Development of *Fusarium* root rot resistant ideotypes in common bean

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Abstract

The study is aiming at developing a superior breeding parent for Fusarium root rot resistance through pyramiding non-allelic resistance genes from five different parents. The rationale is to accumulate and fix resistance quantitative trait loci/genes from different sources of resistance into a single genetic background called *Fusarium* root resistant ideotype. Such ideotypes could be used either directly as improved cultivars or as sources of resistance to improve susceptible market-class varieties. Single-crosses (SC) were developed, evaluated at F_2 and resistant segregants selected. Selected segregants were used to develop double-cross (DC) populations, which were evaluated at the F_3 and the resistant plants selected. The harvested DC F_4 seeds from selected DC F_3 plants will be evaluated and resistant segregants selected. Selected plants will be used in a backcross procedure to fix the accumulated quantitative trait loci/genes. Preliminary results from the evaluations of SC F_2 and DC F_3 showed that both SC F_2 and DC F_3 segregated for highly resistant genotypes suggestive of the effect of cumulated resistance genes from the multiple parents.

Key words: *Fusarium* root rot, *Fusarium solani* f.sp. *phaseoli*, root genotypes, resistant ideotype

Résumé

L'étude vise à développer un parent de reproduction de qualité supérieure pour la résistance à la pourriture racinaire de *Fusarium* par le cumul des gènes de résistance non-alléliques de cinq parents différents. La raison d'être est d'accumuler et de fixer des locus/gènes de traits de résistance quantitatifs provenant de différentes sources de résistance dans un environnement génétique unique appelé idéotyperésistant de *Fusarium*. De tels idéotypes pourraient être utilisés soit directement comme des cultivars améliorés, soit comme des sources de résistance pour améliorer des variétés sensibles de classe du marché. Des croix simples (SC) ont été développées,

évaluées à F_2 et aux ségrégantsrésistants sélectionnés. Les ségrégants sélectionnés ont été utilisés pour développer des populations des croix doubles (DC),qui ont été évaluées à F_3 et aux plantes résistantes sélectionnées. Les graines récoltées des DC F_4 de certaines plantes des croix doubles F_3 seront évaluées et des ségrégants résistants sélectionnés. Les plantes sélectionnées seront utilisées dans une procédure de croisement en retour pour fixer les locus/ gènes de caractère quantitatif accumulés. Les résultats préliminaires des évaluations de F_2 SC et F_3 DC ont montré que F_2SC et F_3 DC ont séparé les génotypes hautement résistants suggestifs de l'effet des gènes de résistance cumulés des parents multiples.

Mots clés: pourriture racinairede *Fusarium, Fusarium solani f.sp. phaseoli*, génotypes des racines, Idéotyperesistant

Background

Bean root rot (BRR), caused by a complex of several different soil-borne fungi (*Pythium* sp, *Fusarium solani* fsp. *phaseoli, Rhizoctonia solani, Macrophomina phaseoli* and *Sclerotium rolfsii*) is very common in bean crops (*Phaseolus vulgaris* L.) that are under stress, i.e., low soil fertility, high humidity, warm to high temperatures, high or low soil moisture, compacted soils, drought, acid soils or soils fertilised with ammonium fertilisers. Yield losses of up to 100% in Uganda (Tusiime, 2003) and 70% in Rwanda (Buruchara *et al*., 2001) due to BRR occurring on susceptible varieties have been reported. The disease has also emerged as the most important constraint to bean production in western Kenya (Otsyula *et al*., 1998), some regions of the Republic of Rwanda and the Democratic Republic of Congo, that neighbour southwestern Uganda (Buruchara *et al*., 2001), and even in Malawi (Snapp *et al*., 2006). Of the BRR, *Fusarium* root rot (FRR), caused by the fungus *Fusarium solani* f.sp. *phaseoli* (FSP), is among the most serious and widespread root rot diseases occurring in most bean fields. Yield losses of up to 84% have been attributed to FRR in susceptible bean cultivars (Park and Tu, 1994). Such a loss undermines the potential of beans as a main source of dietary protein, a food security crop, and as a source of income for many rural poor. The use of cultural practices and fungicides has proven not wholly effective means of control, making genetic resistance the most economic and environmentally-friendly strategy for managing FRR. Several bean lines were identified as sources of resistance to FRR with varying levels of resistance (Mukankusi *et al*., 2010; 2012). The resistance genes in these parents were found to be non-allelic in nature and with additive gene action (Mukankusi

et al., 2011). Combining non-allelic resistance genes from the different parents into a single genetic background has been suggested to result in durable and broad spectrum disease resistance (Pastor-Corrales *et al*., 1998; Obala *et al*., 2012). This study therefore aimed at accumulating and fixing FRR resistance from multiple sources into a single genetic background.

Literature Summary

Previous studies demonstrated that FRR resistance in common bean is conditioned by several genes and that these genes are located at different loci suggesting that stacking these genes into a single genetic background could result in a higher level of resistance than resistance transmitted from single sources and hence, speed up the breeding progress (Mukankusi *et al*., 2011). To validate this assumption, a study was conducted to determine how effective combined resistance from four sources is in improving levels of resistance to FRR in susceptible large-seeded market class bean cultivars (Obala *et al*., 2012). The results of that study showed that resistance accumulated from the four sources was consistently better than resistance from single sources in improving levels of resistance in the susceptible bean cultivars. That study provided strong evidence for the potential of using gene pyramiding to improve levels of resistance to FRR in common bean. However, that study used a segregating non-selected bulk double-cross population as a resistance source. There is need to create immortal sources of FRR resistance, what we are calling FRR resistant ideotypes.

A two step gene pyramiding scheme for developing an ideotype plant has been described (Joshi and Nayak, 2010; Ye and Smith, 2008). The first step called the pedigree, aims at cumulating one copy of all target genes into a single genotype called the root genotype while the second step, called the fixation step, aims at deriving an ideotype from the root genotype by fixing the accumulated genes into a homozygous state. Servin *et al*. (2004), and Ye and Smith (2008) describe three possible procedures for the fixation step. One of these procedures is backcrossing the root genotype to one of the founding parents. The advantage of this procedure is that the probability of obtaining a genotype that is homozygous for the target genes brought about by the recurrent founding parent but heterozygous for the others is high (Ye and Smith, 2008).

Study Description

Six common bean inbred lines, selected on the basis of their superior general combining ability, with moderate levels of

resistance to FRR (R) (Mukankusi *et al*., 2011; Table 1) were used to develop ten R x R single crosses (SC). The SCF_2 together with resistant (M49) and susceptible (CAL96) checks were evaluated for their reaction to FRR under screen house conditions. Randomised complete block design with three replicates was used. Disease symptom severity was scored 28 days after planting using a scale of 1-9, where 1= no disease and 9 = severely diseased (Chaudhary *et al*., 2006). Plants with disease scores of 1 to 3 were considered resistant, selected, transplanted into plastic pots and used to generate five double-cross (DC) populations. Each of the DC populations, also now referred to as the root genotypes, were advanced to the F_3 generation without evaluation owing to limited seed number. The F_3 populations were then evaluated and selected following the same experimental design and procedure as described for the SC F_2. The F_4 seeds for each cross were harvested in bulk and are yet to be evaluated for their reaction to FRR. Resistant plants will be selected and backcrossed to one of the parents following the backcross breeding procedure suggested by Ye and Smith (2008) to fix the resistant genes brought about by the recurrent founding parent. A total of five backcrosses will be performed with selection for FRR resistance at each backcross F_1 generation. F_2-derived families will be generated at the fifth backcross and advanced up to $F_{2:5}$ families. At each generation of selfing, selection for resistance will be done between and within families. Resistant plants within each superior family will be harvested in bulk for further testing.

Research Application

Preliminary results show that despite the moderate levels of resistance to FRR of the parental lines used to generate the crosses, two of the SC F_2 (Table 2) and four of the DC F_3 populations (Table 3) segregated for highly resistant individuals with disease severity score of 1based on a 1-9 disease scale.

Table 1. Parental lines used to develop the root genotypes.

Genotype	Pedigree	No. of R genes	Origin	Reaction to FRR
G685 (G6)	Moncure no.12 (PI182007)	3-5	Mexico	MR
G2333 (G2)	Gentry 21835 Colorado Teopisca/PI311998	3-5	Mexico	MR
MLB-49-89A (M49)	A 240 X Inyumba	2-6	DRC	MR
MLB-48-89A (M48)	A 240 X Inyumba	2-3	DRC	MR
RWR719 (RW)	Cyunyu x Kermes	2-3	CIAT	MR
G4795 (G4)	Porrillo Sintetico	2-9	CIAT	MR

Source: Mukankusi *et al.* (2011).

Table 2. Distribution of resistant x resistant single-cross F_2 populations in reaction to *Fusarium* root rot under screen house condition.

Genotype	No. tested	% selected	% of plants in a given disease score								
			1[s]	2[s]	3[s]	4	5	6	7	8	9
G2 x G4	90	65.6	0.0	43.3	22.2	3.3	0.0	1.1	0.0	1.1	28.9
G2 x M48	204	59.3	0.0	43.1	16.2	1.0	2.0	0.0	0.0	1.0	36.8
G2 x G6	135	80.7	0.0	52.6	28.1	1.5	0.7	0.0	0.0	0.0	17.0
M49 x G6	152	95.4	0.0	87.5	7.9	0.0	0.0	0.0	0.0	0.7	3.9
M49 x G2	155	77.4	1.9	44.5	31.0	6.5	0.0	0.0	0.0	1.3	14.8
M49 x G4	153	85.0	0.7	69.3	15.0	2.0	1.3	0.0	0.0	1.3	10.5
M49 x M48	167	86.2	0.0	68.9	17.4	1.2	0.0	0.0	0.0	1.2	11.4
RW x G2	106	86.8	0.0	58.5	28.3	0.9	0.0	0.0	0.0	0.9	11.3
RW x G4	143	74.1	0.0	46.2	28.0	1.4	2.1	0.7	0.0	0.0	21.7
RW x M48	154	68.2	0.0	48.7	19.5	4.5	1.3	0.0	0.6	1.3	24.0
RW x M49	129	79.8	0.0	62.0	17.8	0.8	0.8	0.0	0.0	0.8	17.8
RW x G6	126	76.2	0.0	53.2	23.0	4.0	3.2	0.0	0.0	0.0	16.7
M49	140		0.0	77.5	14.6	0.7	0.0	0.0	0.0	0.0	0.0
CAL96	87		0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.8	86.2

[s] Plants having these severity scores were selected.

Table 3. Distribution of the resistant x resistant double-cross F_3 populations in reaction to *Fusarium* root rot under screen house condition.

Genotypes	No. tested	% selected	% of plants in a given disease score								
			1[s]	2[s]	3[s]	4	5	6	7	8	9
(RWxM48)x(M49xG4)	357	79.6	0.0	68.6	10.9	2.8	2.0	0.6	0.3	2.2	12.6
(G2xM48)x(RWxG6)	356	80.1	0.3	71.3	8.4	3.7	1.1	0.0	0.8	1.4	12.9
(RWxG4)x(M49xG6)	334	80.5	0.3	71.3	9.0	3.3	0.6	0.3	0.3	1.5	13.5
(G2xG4)x(RWxM49)	343	74.9	0.3	64.1	10.5	3.2	1.5	0.6	0.0	1.2	18.7
(RWxG6)x(M49xG2)	416	85.5	1.2	76.4	8.2	1.7	1.0	0.5	0.5	1.4	9.1
M49	231		0.0	86.6	12.6	0.9	0.0	0.0	0.0	0.0	0.0
CAL96	171		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0

[s] Plants having these severity scores were selected.

Though a small percent of the total plants tested in each of the populations, the presence of segregants with disease score 1 is a possible evidence of the effect of combined FRR resistance genes in these plant types. This possible evidence is supported by the absence of plants with disease score 1 and disease scores above 4 in M49, the resistant check as well as the absence of plants with disease scores below 8 in CAL96, the susceptible check (Tables 2 and 3) suggesting that segregants with disease score 1 in both the SC F_2 and DCF_3 populations could not have arisen due to disease escape. Based on the criteria of plants

with disease score of 1-3 are considered resistant, 59 to 95 % of plants in each of the SC F_2 populations were selected (Table 2) and hybridised to create the five DC populations (root genotypes). In the DC F_3 populations, 74.9 to 85.5% plants from each population were selected (Table 3) and these are to be evaluated to select resistant plants that will be used in the backcross procedure of the fixation step.

From our results so far, it is feasible to develop FRR resistant bean ideotypes although it would require extensive testing. The use of molecular markers tagged to the targeted QTLs would improve the efficiency in selecting for resistance, however, such markers are yet to be developed.

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