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EVALUATING GENETIC ASSOCIATION BETWEEN FUSARIUM AND PYTHIUM ROOT ROTS RESISTANCES IN THE BEAN GENOTYPE RWR 719

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ABSTRACT

Resistance to Fusarium root rot (*Fusarium solani f.s.p phaseoli*) has been reported in common bean (*Phaseolus vulgaris* L.) sources and is usually associated with Pythium root rot resistance. Pythium root rot (*Pythium ultimum var ultimum*) resistance is controlled by a single dominant gene, marked by a SCAR marker $PYAA19_{800}$. It remains unclear whether the inheritance to resistance of these two bean root rots is genetically independent. We evaluated the association of Fusarium root rot resistance with the Pythium root rots resistance gene and/or the molecular marker $PYAA19_{800}$ in genotype RWR 719. Two populations; F_2 and $F_{2:3}$ lines, generated from RWR 719 (resistant) × K132 (susceptible) were respectively screened with *Fusarium solani* and *Pythium ultimum* isolates, and root damages were scored based on the CIAT 1 – 9 scale. Additionally, the $F_{2:3}$ lines were screened with $PYAA19_{800}$. The F_2 segregation ratio deviated from a single gene model for reaction to *Fusarium solani*. The $F_{2:3}$ lines fit the model for a single dominant gene that confers resistance to *Pythium ultimum*. *Fusarium solani* and *Pythium ultimum* resistances were inherited independently. There was lack of association between $PYAA19_{800}$ and *Fusarium solani* resistance, but the $PYAA19_{800}$ was strongly associated with *Pythium ultimum* resistance. This contradicts the assertion of linkage of the two resistances that was deduced based on the joint occurrence of both resistances in the available donor genotypes.

Key Words: Bean root rots resistance, *Fusarium solani*, molecular marker, *Phaseolus vulgaris*, *Pythium ultimum*

RÉSUMÉ

La résistance à la porriture racinaire par suite du *Fusarium solani f.s.p Phaseoli* a été signalée dans les sources du haricot commun (*Phaseolus vulgaris* L.) et communément associée à la résistance de la pourriture racinaire due au *Pythium*. La résistance à la pourriture racinaire due au pithium (*Pythium ultimum var ultimum*) est controlée par un unique gène dominant marqué par un marquer SCAR PYAA19800. Il demeure incertain si l'héritage de la résistance à ces deux types de pourriture racinaire du haricot est génétiquement indépendant. Nous avons évalué l'association des gènes de résistance à la pourriture racinaire due au *Fusarium solani* et au *Pythium ultimum* et/ou le marqueur moléculaire PYAA19800 dans le génotype RWR 719. Deux populations, F2 et F2:3 issues du croisement entre 719 (résistant) × K132 (sensible) ont été respectivement testées avec des isolats de *Fusarium solani* et *Pythium ultimum*, et les dégâts étaient mesurés sur base de l'échelle de cotation de 1 à 9 du CIAT. Additionnellement, F2:3 étaient testées avec PYAA19800. Le rapport de ségrégation de F2 a dévié du modèle unique pour la réaction au *Fusarium solani*. Les lignées F2:3 se sont adaptées au modèle de l'unique gène dominant qui confère la résistance au *Pythium ultimum*. Les résistances au *Fusarium solani* et au *Pythium ultimum* étaient indépendamment héritées. Il n'y avait aucune association entre PYAA 19800 et la résistance au *Fusarium solani*, mais le PYAA19800 était un gène unique dominant avec résistance au *Pythium ultimum*. Ceci contrédit l'assertion du lien de ces deux résistances déduit sur base de l'occurrence combinée de toutes les deux resistances dans les génotypes donneurs disponibles.

Mots Clés: Bean root rots resistance, *Fusarium solani*, molecular marker, *Phaseolus vulgaris*, *Pythium ultimum*

INTRODUCTION

Root rot of bean *(Phaesolus vulgaris* L.*)* is a major constraint to bean production in Uganda, causing total crop failures in some seasons (Spence, 2003; Namayanja *et al.,* 2003; Kakaire, 2008). Bean root rot is caused by a complex of soil-borne pathogens, the most important ones being *Fusarium solani* f. sp. *Phaseoli* and *Pythium ultimum* var. *ultimum* (Rusuku *et al* 1997; Spence, 2003; Tusiime, 2003). Root rot symptoms due to *Fusarium* sp. and *Pythium sp.* include poor seedling establishment, damping-off, uneven growth, chlorosis, premature defoliation, death of severely infected plants, foliar blight or pod rot, and lower yield (Abawi and Pastor-Corrales, 1990; Román-Avilés *et al.,* 2003; Abwai *et al.,* 2006). The two pathogens occur concurrently in farmer's fields (Tusiime, 2003). Management of bean root rot diseases is complicated due to the involvement of multiple soil-borne pathogens, and by the fact that the pathogens produce oospores that are highly persistent in the soil for several years (Abwai *et al.,* 2006). Several management practices are used to control Fusarium and Pythium root rots; seed or soil treatments with selective pesticides, crop rotations, cover crops, seedbed preparations, among other measures, may improve yield or reduce root rot severity (Abwai *et al*., 1985; CIAT, 2003). These measures have not been consistently economical for the resource poor farmers (CIAT, 2003).

Exploiting host plant resistance is considered as the most effective way of managing bean root rots for small-scale farmers (CIAT, 2003; Otsyula *et al.,* 2005). Resistance to *Fusarium solani* is complex and is conditioned by two or more genes (Schneider *et al.,* 2001; Romans-Aviles, 2005; Mukankusi *et al*., 2011), whereas, *Pythium ultimum* resistance is controlled by a single dominant gene, marked by a dominant SCAR marker-PYAA19$_{800}$ (Otsyula *et al*., 2003; Mahuku *et al*., 2005; Otsyula, 2010). The presence of joint resistance to both Fusarium and Pythium root rots have been observed in several resistance sources (Spence, 2003; Tusiime, 2003; Mukankusi, 2008), and it is thought that genes conferring resistance to Pythium root rot may be closely linked or even the same loci (pleiotropic) as those conferring resistance to Fusarium root rot. Quantitative Trait Loci (QTLs) linked to *Fusarium solani* resistance have been mapped on the same chromosome as that on which gene for resistance to *Pythium ultimum* has been found (Romàn-Avilès and Kelly, 2005 and Mahuku *et al*., 2005), however, it is not clearly known whether the two traits are associated. Dickson and Boettger (1977) using one resistant source (Cornell 2114-12) observed low correlations between resistances to Fusarium and Pythium root rots. They however, used mixed cultures of both pathogens which could have affected the evaluation of co-segregation. It is known that when the two pathogens are together, they exhibit synergistic interactions, exaggerating disease severity (Spence, 2003), and since they show similar symptoms, it is difficult to score the severity of both pathogens on the same plant. In this study pure cultures and a molecular marker technique to evaluate any possible genetic link between resistances to *Fusarium solani* and *Pythium ultimum* root rots in the genotype RWR 719, which is resistant to both pathogens were used.

MATERIALS AND METHODS

Genetic plant materials. The population was developed by crossing RWR 719 (R) with K132 (S) in 2008 at National Crop Resources Research Institute Namulonge (NaCRRI). During 2009, the obtained F_1 seeds were planted in pots in the screenhouse at NaCRRI and were bulk harvested. The F_2 population was evaluated for resistance to Fusarium root rots at National Laboratories Research Institute at Kawanda (NALRI), and the F_2 plants were rescued after 21 days, transplanted in pots, and harvested individually. This generated 100 $F_{2:3}$ lines that were screened with both *Pythium ultimum* isolate and the Pythium molecular marker, PYAA19$_{800}$.

Evaluation of populations using *Fusarium solani* isolate. The most pathogenic isolate of *F. Solani f.sp. phaseoli* (*FSP*-3) that had been identified by Mukankusi (2008) and preserved at NALRI was used. To rejuvenate the *FSP*-3 isolate, pure colonies stored on potato dextrose agar (PDA) slants at 5^{O}C were grown on fresh PDA plates for

a period of 21 days and then multiplied on sterilised sorghum seed. The inoculum was prepared following the method described by Mukankusi (2008). One bottle of inoculum (500ml capacity) was added to pre-sterilised sandy-clay-loam soil in each tray (0.74 x 0.42 x 0.115m^3). A susceptible line, K132, was then planted in each tray for up to 28 days, when it was uprooted. This was done to test the pathogenicity of the *FSP*-3 isolate and to increase disease pressure in the soil before planting the test materials. To ensure uniform levels of inoculum in all trays, soil was removed from all the trays, remixed together and redistributed equally into the trays prior to planting the test populations. The test populations (F_2 seeds) were then planted in four wooden trays containing mature *FSP*-3 inoculum. Each tray was planted with 8 rows of the test material and 2 rows of each of the resistant (RWR 719) and susceptible (K132) parents. The trays were placed on a raised wire mess in the screenhouse and watered four times a week (Mukankusi, 2008).

Reactions to disease were assessed 28 days after planting by washing the below- ground parts of the plant (hypocotyls and roots) under running tap water, and scoring the reaction according to the C1AT 1-9 scale (Abawi and Pastor-Corrales,1990). In this scale; 1 = no visible symptoms 3 = light discolouration either without necrotic lesions or with approximately 10% of the hypocotyl and root tissue covered with lesions; 5 = approximately 25% of the hypocotyl and root tissue covered with lesions though the tissues remain firm, with some deterioration of the root system; 7 = approximately 50% of the hypocotyls and root tissues covered with lesions combined with considerable softening, rotting, and reduction of root system; and 9 = approximately 75% or more of the hypocotyls and root tissues affected with advanced stages of rotting, combined with severe reduction in the root system.

Evaluation of populations using the *Pythium ultimum* isolate. The pathogenic isolate of *Pythium ultimum* var.*ultimum* (MS 61), which had previously been isolated and preserved at CIAT-Kawanda (Mukalazi *et al.*, 2001), was used for this study. A total of 10 seeds of each of 100 $F_{2:3}$ families from RWR x K132 cross were planted in wooden trays containing soil infested with the MS 61 isolate. A tray consisted of two checks and 20 $F_{2:3}$ families; each planted in short rows of five plants per family. The experiment was laid out in a randomised complete block design with two replications of five trays per replication. Germinated seedlings were watered twice a day for three weeks to provide favorable conditions for infection, establishment and development of the fungus (Mukankusi, 2008). Individual seedlings were uprooted after 3 weeks, washed in tap water, and scored according to the CIAT 1-9 scale (Van Shcoonhoven and Pastor-Corrales, 1987). Plants with limited or no symptoms (score 1-3) were rated as being resistant, and the rest of the plants as susceptible. The average score for each of the 100 $F_{2;3}$ families was obtained, and the class frequencies were tested for goodness of fit to the theoretical ratios, using the chi-square test. The correlation between the average scores for Pythium root rot and the Fusarium root rot phenotypic scores was evaluated.

Evaluation of populations with the *Pythium* SCAR marker $PYAA19_{800}$. This experiment was conducted to investigate the association of the *Pythium* molecular marker ($PYAA19_{800}$) with *F. solani and P. ultimum* resistances. Young trifoliate leaves were sampled from six plants for each of the 100 $F_{2:3}$ families described, and the extracted DNA from the plants in the same family was later bulked since each family was derived from a single F_2 plant. Six plants were sampled in order to have a high probability of capturing at least one allele from the resistant parent in each segregating family. One half of the $F_{2:3}$ families were expected to segregate (1RR: 2Rr: 1rr). Total genomic DNA was extracted using the procedure described by Mahuku (2004). Extracted DNA was quantified with a DQ200 fluorometer following the manufacturer's instructions, and DNA from each sample was then diluted based on concentration of each sample to 20 or 80 $ng{\cdot}ml^{-1}$ for polymerase chain reactions (PCR). The PCR master mix was constituted to consist of 0,2 mM of dNTPs, 2mM $MgCl_2$, 1µ/25µl of Taq Polymerase, 1X PCR Buffer and 0,4 µM of each primer.

The sequences of the primers used were: 5' - TTA GGC ATG TTA ATT CAC GTT GG-3' and 5'- TGA GGC GTG TAA GGT CAG AG- 3' (CIAT, 2008). The 25µl-reaction PCR volume was subjected to 34 amplification cycles in a BIO RAD MyCycler thermal cycler. The amplification consisted of one cycle at 94°C for 5 minutes, followed by 34 cycles of: denaturation at 94ºC for 40 seconds, annealing at 63°C for 40 seconds, and extension at 72°C for 40 seconds for 34 cycles, followed by a final extension for 7 minutes at 72°C and a holding temperature of 4°C. The amplified products were separated according to size, using 1.2% agarose gel in a 0.5X TBE buffer at a voltage of 100V for 45 minutes, and then stained with ethidium bromide (0.5µg/ml) to be visualised under ultraviolet light and photographed before scoring. Bands were scored as 1 or 0 for presence or absence of a specific DNA fragment. This data was compared with FSP phenotypic scores to detect a possible association.

Association analyses. A genetic linkage test was performed based on chi-square test of independence, using phenotypic classes (R, S) for Fusarium and Pythium root rots in order to assess the association of resistances. This result was supported by running a correlation analysis in Genstat (Genstat, 2010). To assess the association of the $PYAA19_{800}$ marker locus with the *Fusarium solani* phenotypic class (R or S), a chi-square test of independence was performed. A regression analysis of $PYAA19_{800}$ against Fusarium root rot score was performed in Genstat to estimate the magnitude of the marker effect.

RESULTS

When F_2 populations of RWR 719 x K132 cross were inoculated with *Fusarium solani* isolate, the 100 F_2 plants segregated into 87 resistant : 13 susceptible. Segregation ratio for the F_2 population deviated from a 3: 1 gene model ($\chi 2$ = 7.68, P = 0.006) (Table 1). For the 100 $F_{2:3}$ lines of the same cross, the segregation ratio was 72 resistant : 28 susceptible for reaction to *Pythium ultimum* which fit the 3:1 model for a single dominant gene ($\chi 2$ = 0.48, *P* = 0.49) (Table 1). The chi-square test of independence for resistance to both *Fusarium Solani* and *Pythium ultimum* in RWR 719 × K132 cross showed no significant relationship between the two pathogens ($\chi 2$ = 4.96, P = 0.29) (Table 2). These results were further supported by a correlation analysis that displayed no significant association between the two traits (R^2 = 0.007, P = 0.39, Fig. 1). When 100 $F_{2:3}$ lines were screened with $PYAA19_{800}$ (a dominant SCAR marker), 78 lines produced bands that indicated the presence of the *Pythium* resistance gene and 22 lines showed no band, indicating absence of the gene (Fig. 2). The marker scores fit a 3:1 ratio ($\chi 2$ = 0.48, *P* = 0.49) for a dominant marker indicating normal segregation in a simple Mendelian ratio (Table 3). Results of the test of independence showed no significant association of Fusarium root rot resistance with the Pythium marker $PYAA19_{800}$ ($\chi 2$ = 0.06, P =

TABLE 1. Chi-square goodness of fit for reactions to *Pythium ultimum* and *Fusarium solani* root rots of populations from RWR 719 (R) × K132 (S) cross

Reaction+	Number of lines				$\chi 2$ (3:1 ratio)	P (df =1)
	Observed		Expected			
	R	S	R	S		
*Pythium ultimum**	72	28	3	1	0.48^{ns}	0.49
Fusarium solani#	87	13	3	1	7.68**	0.006

+R = resistant (score 1-4.9; S = susceptible (score >5). *Each replication contained 5 plants per $F_{2:3}$ lines and was repeated two times. # Individual F_2 plant score, not replicated. All scores based on 1-9 scale

Note: the 100 populations constituted only plants that survived FSP screen and produced $F_{2:3}$ lines, thus this population tended to have more number of FSP resistant plants than the susceptible ones

TABLE 2. Test of independence for the *Fusarium solani* f. sp. *Phaseoli* and *Pythium ultimum* resistance genes in F_2 and $F_{2:3}$ populations from RWR 719 (R) × K132 (S)

*Pythium ultimum** \ *Fusarium solani* #	Observed (Expected)			Total
	R	I	S	
S	22 (22.12)	2 (2.24)	4 (3.64)	28
I	22 (21.33)	4 (2.16)	1 (3.51)	27
R	35 (35.55)	2 (3.60)	8 (5.85)	45
Total	79	8	13	100
$\chi 2$				4.96^{ns}
prob.(df = 4)				0.29

R = resistant (score 1-3.9); I = intermediate (score 4-4.9); S = susceptible (score >5). *Each replication contained 5 plants per $F_{2:3}$ lines and was repeated two times; #Individual F_2 plant score, not replicated. All scores based on 1-9 scale. ^{ns}No significant departure from the hypothesis of independent segregation

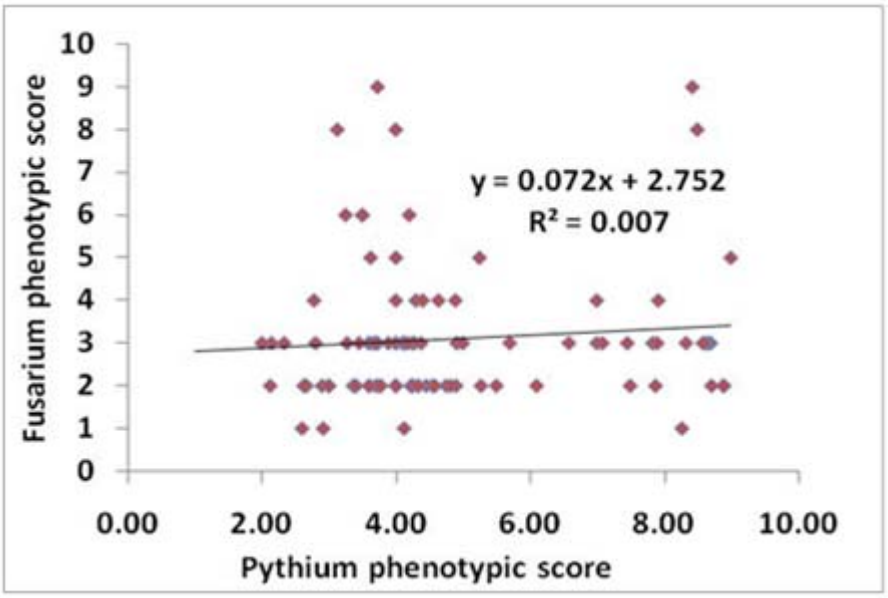
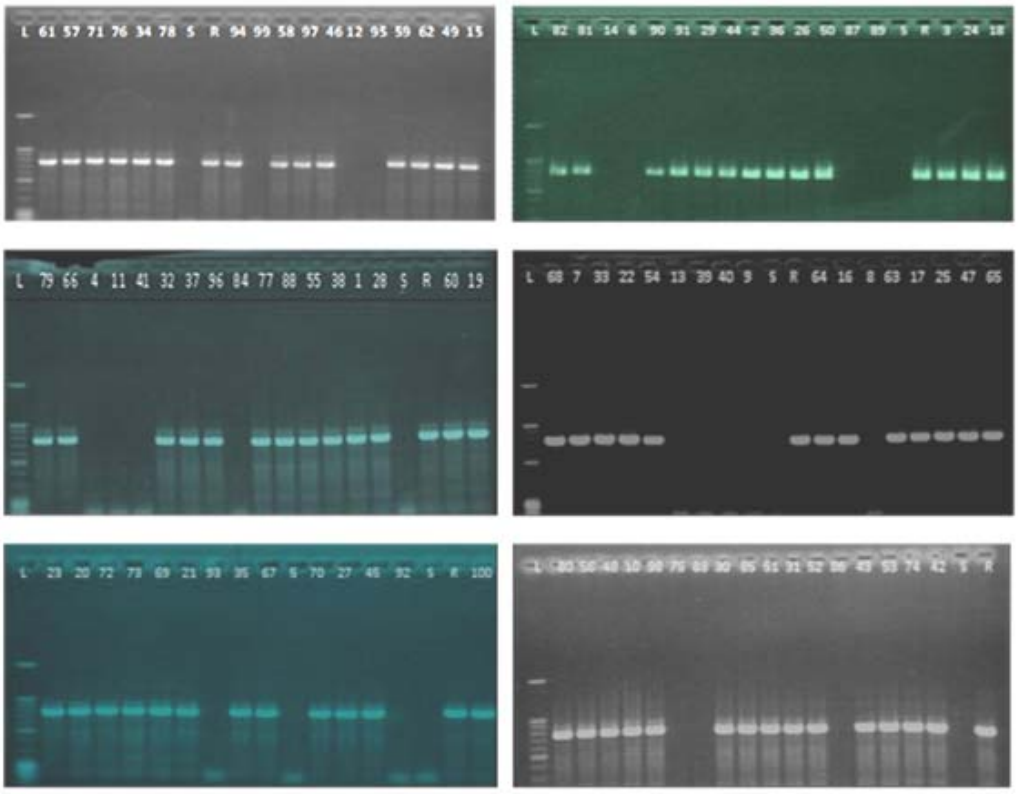


Figure 1. Scatter plots showing the relationship between *Fusarium* and *Pythium* resistances in the genotype RWR 719.

TABLE 3. Chi-square goodness of fit for the segregation of the SCAR marker $PYAA19_{800}$ in 100 $F_{2:3}$ bean lines from RWR 719 (R) × K132 (S) cross

Population	Genotype	Number of lines*		$\chi 2$ (3:1 ratio)	P (df =1)
		+	-		
RWR 719 x K132	($F_{2:3}$ lines) PYAA19	78	22	0.48^{ns}	0.49

*Number of lines showing presence or absence of the band that matches that of the resistant parent (R). ^{ns}No significant departure from 3:1 ratio



Key: L = ladder, S = susceptible check, R = resistant check.

Figure 2 Electrophoresis pictures for the screening of 100 $F_{2:3}$ lines from RWR719 (R) x K132 (S) cross with the dominant SCAR molecular marker $PYAA19_{800}$.

TABLE 4. Chi-square test of independence of marker $PYAA19_{800}$ from *Fusarium* and *Pythium* resistances in $F_{2:3}$ lines from RWR 719 (R) x K132 (S) cross

Reaction[++]	Category[+]	Number of lines			
		Fusarium [#]		Pythium[##]	
Marker $PYAA19_{800}$		Obs.	Exp.	Obs.	Exp.
1	R	68	67.86	72	56.16
1	S	10	10.14	6	21.84
0	R	19	19.14	0	15.84
0	S	3	2.86	22	6.16
	Total	100	100	100	100
	$\chi 2$	0.06[ns]		72.52***	
	Prob (df = 2)	0.97		1.78E-16	

[+]FSP and Pythium phenotypic classes, grouped as R = resistant (score 1-3.9); I = intermediate (score 4-4.9); S = susceptible (score >5). [++] Marker scores of 0 and 1 correspond to absence and presence of the band corresponding to that of the resistant parents. [##]Each replication contained 5 plants per $F_{2:3}$ lines and was repeated twice. [#]Individual F_2 plant score, not replicated. All scores based on a 1-9 scale. [ns]No significant departure from the hypothesis that the marker and the phenotypes are independent. ***Significant departure from the hypothesis that the marker and the phenotypes are independent

TABLE 5. Regression of *Fusarium solani* and *Pythium ultimum* scores in populations from RWR 719 (R) x K132 (S) cross onto the marker $PYAA19_{800}$ score

Source	d.f.	Fusarium+		Pythium++	
		m.s.	F pr.	m.s.	F pr.
Marker $PYAA19_{800}$	1.00	1.34^{ns}	0.48	283.50***	2.69E-33
Residual	98	2.63		0.85	
Total	99	2.62		3.71	
R^2		0.005^{ns}		0.77***	

+100 F2 plant score regressed on marker PYAA19 score, experiment not replicated
++ 100 F2:3 mean scores regressed on marker PYAA19 score, experiment replicated 2 times
*** Regression significant at P < 0.001; ns Regression not significant

72.5), although the marker was strongly associated with Pythium root rot resistance ($\chi 2$ = 0.97, P = 1.78E-16) (Table 4). The results from the test of independence were very consistent with the regression analysis (Table 5).

DISCUSSION

The current study revealed that Pythium root rot resistance was conditioned by a single dominant gene. Also, based on earlier studies, Hassan *et al.* (1971), Boomstra and Bliss (1977), Faria (1983) Schneider *et al.* (2001), Romans-Aviles and Kelly (2005), Mukankusi (2008) among others reported that two or more genes govern *Fusarium* resistance which closely agree with the present finding. Consistent with Buruchara *et al.* (2001, Unpubl.), Otsyuala *et al.* (2003), and Otsyuala (2010).

Results from the study, are at variance with previous findings (Tusiime, 2003; Spence, 2003; Mukankusi, 2008) indicated that Fusarium and Pythium root rots resistances in RWR719 were independently inherited. It is probable that the joint resistance in genotype RWR 719 was a result of field selection of materials with both Fusarium and Pythium root rots resistances since the two pathogens frequently occur together in the field.

Genetic independence of the inheritance of resistance for the two bean roots rots is in line with findings by Dickson and Boettger (1977) who observed low correlations between the two traits. Additionally, marker analysis revealed lack of association between *Fusarium solani* resistance and the Pythium marker $PYAA19_{800}$. As expected, Pythium marker $PYAA19_{800}$ was closely associated with *Pythium ultimum* resistance in RWR 719, explaining over 77% of phenotypic variability, and confirming its suitability for use is breeding for *P. ultimim* resistance. Mahuku *et al.* (2005) had reported an association of $PYAA19_{800}$ with *Pythium ultimum* resistance in RWR 719, MLB 49-89A and AND 1062, located 1.5cM from the resistance gene.

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