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Full Length Research Paper

Inheritance of fusarium wilts (*Fusarium oxysporum* F. sp. *phaseoli*) resistance in climbing beans

Augustine Musoni[1]*, Paul Kimani[2], R. D. Narla[2], Robin Buruchara[3] and James Kelly[4]

[1]Institute of Sciences and Agricultural Research of Rwanda (ISAR), P. O. Box 82, Nyagatare, Rwanda.
[2]College of Agriculture and Veterinary Sciences, University of Nairobi, P. O. Box 29053, Nairobi, Kenya.
[3]CIAT-Pan Africa Bean Research Alliance, P. O. Box 6247, Kampala.
[4]Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824.

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Fusarium wilt, caused by *Fusarium oxysporum* F. sp. *phaseoli* caused growers to abandon the most popular climbing bean cultivar, Umubano (G2333) in Rwanda. The present objective was to determine the nature of inheritance of fusarium wilt resistance and recommend a breeding strategy to introduce resistance into susceptible cultivars. Two cultivars, vuninkingi (G685) and flora were donors of resistance to fusarium wilt whereas G2333 was highly susceptible. Injured root tips of 10-day old seedlings of the parents, progenies of F_1 and F_2 (G2333 × G685) and (G2333 × Flora), backcrosses F_2 (G685 × Flora) were inoculated with 10^6 conidia ml^{-1} of Rwandan isolate of *F. oxysporum* F. sp phaseoli (FOP-RW2) in a glasshouse. The disease severity was rated 28 days later using the CIAT scale of 1 - 9, where 1 - 3 represent resistant, 4 - 6 tolerant and 7 - 9 susceptible reactions. The chi-square analysis was performed to determine the Mendelian segregation ratios of resistant and susceptibility among the inoculated progenies. The F_1 and the backcross progenies to the resistant parents segregated in the ratio of 1:0 as did the F_2 population (G685 × Flora). The F_2 progenies segregated in the ratio of 3:1. The backcross progenies to the susceptible parent segregated in the ratio of 1:1. Resistance to fusarium wilt is conditioned by a single highly heritable major dominant gene. The resistance can be achieved by backcross breeding.

Key words: Backcross breeding, heritability, major gene, fusrium wilt, Mendelian ratios, Umubano cultivar.

INTRODUCTION

Fusarium wilt disease of beans (*Phaseolus vulgaris* L.) is caused by a soil-borne fungal pathogen, *Fusarium oxysporum* F. sp. phaseoli (*Fop*) that causes up to 100% crop damage on susceptible cultivars under ideal conditions (Buruchara et al., 1999). The epidemics of the disease in Rwanda in the late 1980s caused total crop failure that resulted in the abandonment by farmers of the most popularly adopted climbing bean cultivar, Umubano (G2333). The cultivar that had been released five years earlier had attained over 80% but fell susceptible to the disease.

The use of improved resistant cultivars is known to be the most feasible, sustainable and cost-effective control strategy of the crop diseases, especially among the land-scarce and resource poor farmers (Devries et al., 2001). The alternative management practices such as fallow and crop rotation are prevented by diminished cropland of less than 1 ha per household (Chrispeels et al., 2003). Biochemical solutions (Mutitu, 1989) are expensive and least understood or acceptable by the small holder farmers. They are more likely to destabilize production environment in the long run (Devries et al., 2001).

While the sources of resistance against fusarium wilt such as vuninkingi (G685) and Flor de Mayo (Flora) were identified, there have been no inheritance studies of resistance against the disease in Rwanda or Africa. This complicates the breeding strategy for the improvement of susceptible commercial cultivars such as G2333. The objective of the current study was therefore to determine the nature of inheritance of fusarium wilt resistance in common beans and to suggest the most effective breeding strategy to improve the resistance of the susceptible commercial climbing beans against the disease.

*Corresponding author. E-mail: afmusoni2@yahoo.com. Tel: 565079, +250 08747932, 03703218.

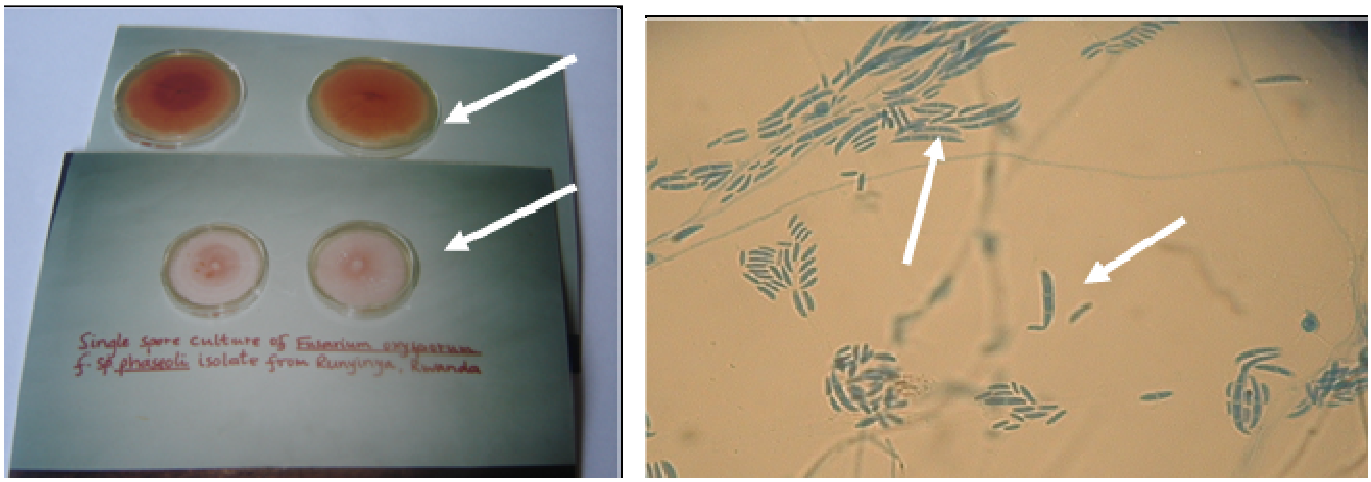

Plate 1. Bottom and surface view (left, arrowed); single and mass of elongate and curved spores (arrowed, right) of *F. oxysporum* F. sp. phaseoli cultures.

MATERIALS AND METHODS

Vuninkingi and Flora were used as donors of resistance to *Fop* to the susceptible parent Umubano in pair-wise crosses in the green house at Kabete Field Station, University of Nairobi. The plants were grown in 30 cm diameter plastic pots, filled with a mixture of sterilized loam soil and manure at a ratio of 4:1 (Buruchara and Camaco, 1999). Crosses were made after emasculating young flower buds of the female plants. Mature anther heads from the flowers of male parents were brushed to the exposed stigma of the freshly opened young flower buds of the vigorous and disease-free female plants in the greenhouse (Buishand, 1956; Temple et al., 1998). Crossing operations were made using ethanol-sterilized forceps. A portion of the F_1 seed was saved and the rest was used to raise backcross and F_2 progenies of the same crosses. Before the disease inoculation, the parents, F_1, F_2 and backcross progenies were planted in sterilized sand (80%), loam soil (20%) mixture treated with 3 g of diammonium phosphate fertilizer per pot inside the plastic pots arranged in a completely randomized block design with 2 replications of 25, 25, 125 and 100 plants per replicate for the above respective parents and progenies. They were watered daily and fresh fertilizer dose was renewed weekly.

Isolation and culture preparation

One-centimeter long segments of the hypocotyl of diseased plants of Umubano cultivar were washed, chopped, peeled and split open and were sterilized into 2% sodium hypochlorite ($NaHClO_2$) solution for 2 min, rinsed twice in distilled water and were dried using sterile blotting paper under a lamina flow hood environment. Portions of infected tissue were transferred into a 9 cm -diameter sterilized Petri dishes containing cooled sterile potato dextrose agar (PDA) that was inoculated with streptomycin sulphate at 0.4 g/l to minimize bacterial contamination (Ribeiro et al., 1979; Mutitu, 1989). They were incubated at 24°C for 4 days. Portions of the agar plate bearing spreading colonies were then transferred onto fresh Petri dishes plated sterile tap water agar (TWA) and were incubated again. Observing the pinkish growing colony on TWA plate (Plate 1 left) under a stereomicroscope identified single, elongate, curved and septated spores (Plate 1 right) germinating macroconidia were isolated and transferred onto fresh PDA/streptomycin plated Petri dishes by help of sterilized wire loop containing a sterile water film. They were incubated for 14 days at 24°C.

Inoculum preparation

By adding 20 ml of distilled water to mature cultures (Figure 1) on each plate and scrapping gently its surface using the curved end of a sterile glass rod, a uniform suspension of the single spore culture was made. The contents of all the Petri dishes were aseptically strained through a double layer of cheesecloth into a beaker. The suspension was centrifuged at × 5000 revolutions per min for 10 min and the spore pellet rinsed twice in distilled water. The pellet was then uniformly mixed with 50 ml of distilled water and the initial spore concentration, C1, and the final volume, V2, containing the standard 10^6 conidia per milliliter (Robin and Camacho, 1999) was determined using the hemacytometer method.

Inoculation

The method of inoculating roots was a slight modification of the root-dip and transplanting technique model practiced by Ribeiro et al. (1979) and Buruchara et al. (1999). The young roots and root hairs of intact, well-watered, ten-day old seedlings were crack-injured by incomplete uprooting of the seedling. Tips of lateral root hairs around the loose hypocotyls were exposed and injured using new scalpel blades. 20 ml of the uniform 10^6 conidia per milliliter suspension was poured around the plant so as to flood the root region within 2 cm radius of the hypocotyls. The inoculated plants were firmly secured back into the soil. This was meant to minimize plant shock associated with transplanting large numbers of seedlings**.**

Watering of the plants was resumed 24 h after inoculation. The soil moisture regime was maintained at about field capacity throughout the study (Mutitu, 1989). The pots were supplied with 3 g ml^{-1} of fertilizer (NPK 17:17:17) at an interval of 7 days.

Assessment of fusarium wilt reaction

The appearance of chlorosis, wilting, and necrosis of leaves, branches or stems of all the plants were monitored daily and the final disease rating was done after 28 days (Buruchara and Camacho, 1999) (Plate 2 right). Random samples of 20 plants from each population were uprooted, washed and split open with a scapel blade. The external and internal intensity of the brown discoloration was observed and its maximum upward spread along

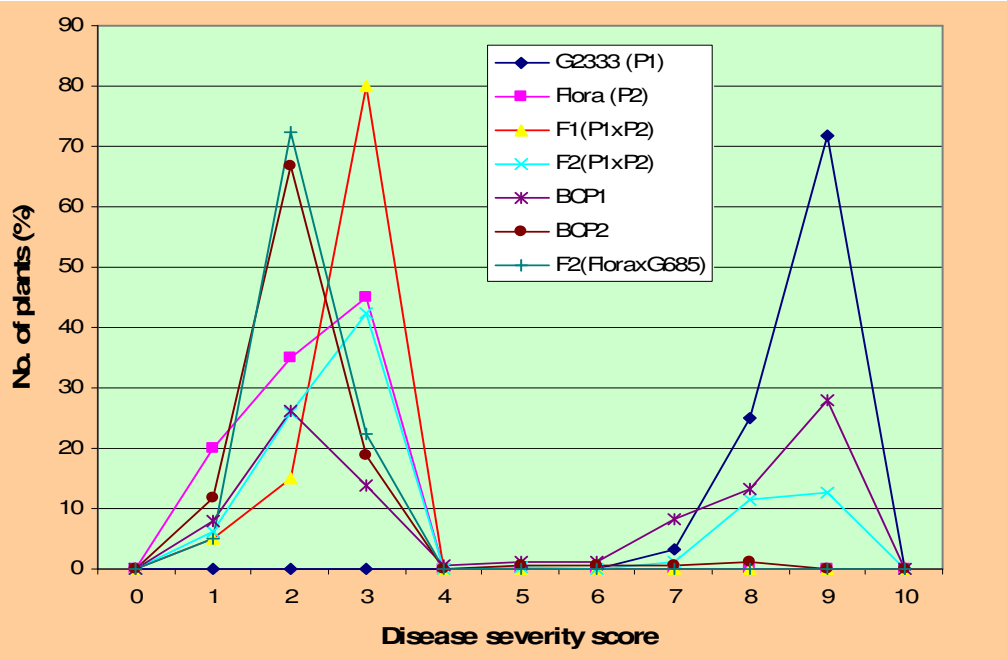

Figure 1. Distributions of *Fusarium oxysporum* f. sp. phaseoli reaction among G2333 × Flora lines and progenies after artificial inoculation in screenhouse at Kabete.

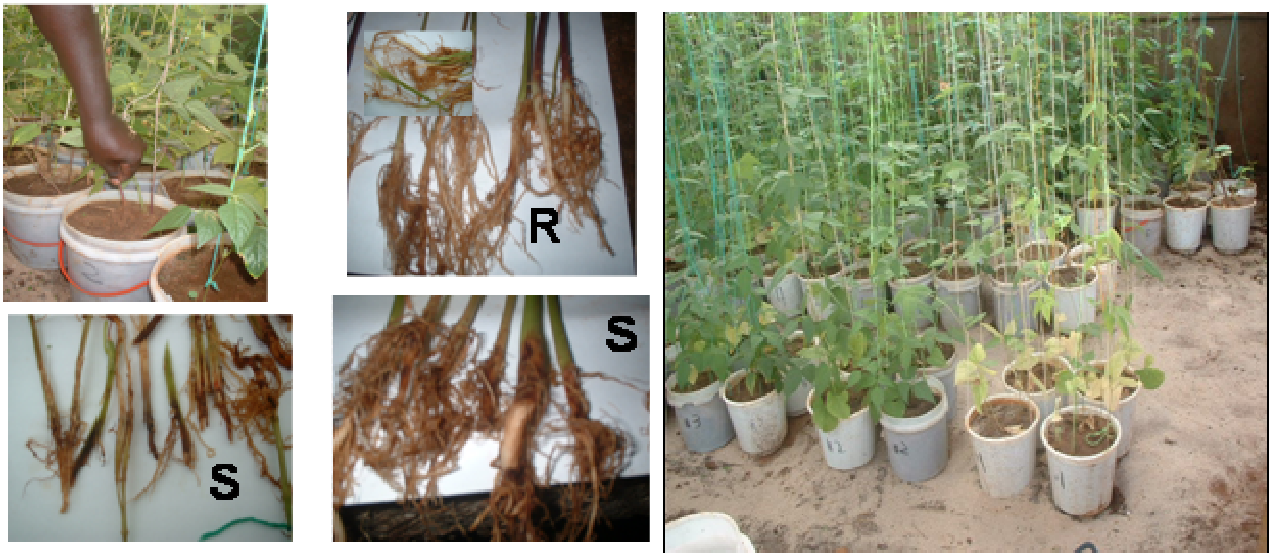

Plate 2. Observed external and internal browning and chlorotic root damage (left); yellowing leaf symptoms (S) on susceptible parent, Umubano, and normal resistant (R) reaction on Vuninkingi donor parent after artificial inoculation with *F. oxysporum* f. sp. phaseoli isolate at Kabete, 28 days after inoculation (DAI).

along the xylem vessels from the hypocotyls was measured (Plate 2 left). The disease assessment was done using a CIAT severity score scale of 1 - 9, where 1 - 3 represents resistant reaction with no more than 10% of total foliage being chloritic or wilted and no or light vascular browning; 4 - 6 stands for intermediate reaction (11 - 50%) foliar chlorosis and wilting with light to slightly severe vascular discoloration and the scores of 7 - 9 representing susceptible reaction manifested by more than 50 to 100% foliar yellowing, wilting, defoliation, severe to very severe discoloration or complete death of the plant (van Schoonhoven and Pastor-Corrales, 1987). The chi-square analysis was performed to determine the Mendelian segregation ratios of resistant and susceptibility among the inoculated progenies. Means, variances of individual plants of disease, severity scores from each population were computed and were used to calculate environmental variance, V_E and to perform heritability analysis by the method recommended by Kearsey et al. (1996) as

Table 1a. Chi-square analysis and segregation ratios among parents, F_1, F_2 and backcross progenies after inoculation with *F. oxysporum* F. sp. *phaseoli* in screenhouse at Kabete.

Cultivar or cross	Plants screened	Observed segregation (R:S)	Tested ratio (R: S)	X^2 observed	Probability
G 2333 (P_1)	32	0:32	0:1	-	-
G 685 (P_2)	38	38:0	1:0	-	-
F_1 (G 2333 × G685)	39	39:0	1:0	-	-
F_2 (G 2333 × G685)	256	194:62	3:1	0.109**	0.70 - 0.80
(G 2333 × G 685) × P_1	180	89:91	1:1	0.050***	0.80 -0.90
(G 2333 × G 685) × P_2	178	177:1	1:0	0.013***	0.90 - 0.95
F2 (Flora × G685)	40	40:0	1:0	-	-

** Differences statistically significant; *** Differences highly significant.

Table 1b. Number of resistant or susceptible plants or progenies after artificial inoculation with *F. oxysporum* f. sp. *phaseoli* isolate in a screenhouse at Kabete.

Population	REP I		REP II		Total no. of plants		
	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Total
G2333 (P_1)	0	16	0	16	0	32	32
G685 (P_2)	20	0	18	0	38	0	38
F_1(P_1 × P_2)	19	0	20	0	39	0	39
F_2(P_1 × P_2)	96	30	98	32	194	62	256
BCP_1	47	43	42	48	89	91	180
BCP_2	87	1	90	0	177	1	178
G685×Flora	20	0	20	0	40	0	40
G2333 (P_1)	0	16	0	16	0	32	32
Flora (P_2)	20	0	20	0	40	0	40
F1(P_1 × P_2)	20	0	20	0	40	0	40
F2(P_1 × P_2)	100	30	93	37	193	67	260
BCP_1	52	38	34	56	86	94	180
BCP_2	86	4	89	1	175	5	180
Flora × G685	20	0	20	0	40	0	40
Total	587	178	564	206	1151	384	1535
	770		765		1535		

follows:

The environmental variance (V_E) was computed by obtaining the ratio of the total sum of the squares (SS) to combined df of the parental homozygous lines, using the formulae:

$SS = \sum X^2 - C.F. = \sum X2 - (\sum X)^2/N$; and

V_E = SS *G2333* + SS *G685* / DF G2333*DFG685

where X = individual disease score; C.F = correction factor; N = total number of plants evaluated in each parent.

Heritability (H^2_{bs}) was estimated from the values of computed V_E and variance of the F_2 populations as follows:

$V_P = V_G + V_E$; or, $V_G = V_P - V_E$; and

$H^2_{bs} = V_G/ (V_G + V_E)$,

Where, V_P = phenotypic variance, given by the respective variance of the F_2 progenies; V_G= the genetic component of the variance; V_E = the variance due to environmental factors; and H^2_{bs} = the broad sense heritability of the resistant trait.

RESULTS

All plants of Umubano were susceptible with the severity score ranging between 7 and 9. The plants of the donor parents, G685 and Flora and all the F_1 progenies showed resistant reaction of 1, 2 or 3 scores. About three-quarters of the F_2 progenies of both crosses were resistant and one-quarter was susceptible. The backcross progenies of Umubano segregated in the ratio of 1: 1 for the resistant and susceptible reaction, while the backcrosses of both donor parents were all significantly resistant to fusarium wilt (Tables 1a, b and 2).

The mean severity scores of the F_1 of G2333 × G685 and of G2333 × Flora were 2.2 and 2.8, respectively. They were very close to the mean severity score of the donor parents of 1.6 and 2.3, respectively, within the resistant severity bracket. The mean severity score of the F_2 progenies (3.9 and 4.0) of the same crosses were higher but closer to their donor parents' and much lower

Table 2. Chi-square analysis for G2333, Flora, their F_1, F_2 and backcross progenies and segregation ratios after inoculation with *F. oxysporum* F. sp. *phaseoli* in screenhouse at Kabete

Cultivar or cross	Plants screened	Observed segregation (R:S)	Tested ratio (R: S)	X^2 observed	Probability
G 2333 (P_1)	32	0:32	0:1	-	-
Flora (P_2)	40	40:0	1:0	-	-
F_1 (G 2333 × G685)	40	40:0	1:0	-	-
F_2 (G 2333 × G685)	260	193:67	3:1	0.067**	0.70 - 0.80
(G 2333 × G 685) × P_1	180	86:94	1:1	0.450**	0.50 - 0.70
(G 2333 × G 685) × P_2	180	175:5	1:0	0.113**	0.70 -0.80
F2 (Flora × G685)	40	40 : 0	1:0	-	-

Table 3. Analysis of variance and heritability of resistance against fusarium wilt obtained from the G2333 × G685 and G2333 × Flora crosses.

Source	df	SS	Variance	V_P	V_G	V_E	H^2_{bs}	H^2_{bs} (%)
G2333 × G685								
G2333	31	13.22	0.462					
G685	37	20.84	0.563	7.766	7.736	0.0297	0.996	99.6
Total	1147	34.06	0.0297					
G2333 × Flora								
G2333	31	8.875	0.286					
Flora	39	23.50	0.603	7.127	7.100	0.0268	0.996	99.6
Total	1209	32.375	0..268					

than for the susceptible parent of 8.7 (Figure 1).

Variance was lowest and less than 1 in the resistant and susceptible parents and in the F_1 as well as F_2 progenies of Flora × G685; meaning these populations were more uniform. It was highest in the F_2 progenies of G2333 × G685 (7.8) and of G2333 × Flora (7.1) and of both backcrosses to the susceptible parent, G2333. The heritability of the *Fop* resistance was found to be 99.6% in both the G2333 × G685 and G2333 × Flora crosses (Table 3).

DISCUSSION

The observed generation means of disease severity confirmed that G2333 (score 8.7) was very susceptible to infection by *F. oxysporum*. In contrast, G685 and Flora showed resistant reactions. The results closely agree with the severity rating of 9 for G2333, of 1.3 and 2.6 for G685 and Flora, respectively, as observed by Buruchara and Camacho (1999). The mean disease severity score of the F_1 progenies was 2.2 and 2.8 for the G2333 × G685 and G2333 × Flora crosses, which showed that the F_1 progeny plants inherited resistance to fusarium wilt from the donor parents and expression was completely dominant.

The closer leaning and association of the disease severity reaction of the F_1 and the F_2 progenies towards the resistant parent suggests that the gene that conditions resistance against fusarium wilt is dominant to its recessive allele. It further means that its action is more influenced by non-additive (dominance) than additive effects (Allard, 1960; Kearsey et al., 1996; Griffiths et al., 1998).

Genetic variance

The computed variances were highest in the F_2 progenies of both G2333 × G685 and G2333 × Flora crosses and among progenies of the back-cross with the susceptible parent; meaning that their segregation which ranges within the resistant and susceptibility reactions were more divergent as expected. Conversely, the parental lines and all the F_1 progenies were more genetically uniform as proved by the smaller values of their variances. The broad and narrow spreads of disease reaction among the F_2 and F_1 progeny populations, respectively, was as expected in a qualitative mode of inheritance.

Heritability

The observed high heritability value of nearly 100% is a further proof that the resistance against fusarium wilt is inherited qualitatively rather than quantitatively. Its ex-

pression is least affected by the surroundings, as shown by very low values of the environmental variance, V_E of nearly 0 and high genetic variances V_G (about 1) (Table 3).

Major dominant gene

The probability values (Tables 1a and 2) indicates that there are no significant differences between the observed and the expected segregation ratios of resistant and susceptible plants. The F_2 generation progenies of (G2333 × G685) and G2333 × Flora segregated in the expected ratio of 3 resistant: 1 susceptible for a dominant gene. The segregation pattern among the F_1 backcross progenies of the same (G2333 × G685) × G2333 and (G2333 × Flora) × G2333 was 1 resistant: 1 susceptible. Similarly, the segregation of the backcross F_1 (G2333 × G685) × G685 and of F_1 (G2333 × Flora) × Flora progenies were in agreement with the expected Mendelian ratio of 1 resistant: 0 susceptible for a major dominant gene. The results of these populations confirm that the inheritance of resistance against fusarium wilt disease is determined by a major dominant gene. All the F_2 progenies of the Flora x G685 (cross between resistant donor parents) were found to be highly resistant to the fusarium wilt attack with a mean severity score of 2.0. It compared closely with the resistant reaction that was recorded for either of the parents. The result suggests the presence of a single locus conditioning resistance in both cultivars in which the gene that conditions the resistance is completely dominant to its recessive allele.

Ribeiro and Hagedorn (1979) observed similar segregation pattern of 3:1 among the F_2 generation progenies and of 1:1 and 1:0 in the backcross progenies in bush beans when they inoculated the progenies raised from crosses of the resistant parents Tenderette, Pintado and Early Gallatin with the susceptible Bush Blue Lake 274 (BBL 274) with a Brazilian isolate of *Fop.* He designnated a single completely dominant gene, *Fop 1* and its recessive allele *Fop$^+$ 1* as being the alleles that confer the resistance or susceptibility to *Fop* attack in *phaseolus* beans.

They, however, observed a modified monohybrid segregation pattern of 1:2:1 among the F_2 progenies of the susceptible × resistant parents when the inoculation was repeated using the North American race of the same organism. They suggested the presence of another resistant gene; they coded as *Fop 2*, that was incompletely dominant to its recessive allele, *Fop$^+$ 2.*

Considering the close similarity of the levels of disease incitement in the same cultivars (G2333, G685 and Flora) caused by the current Rwandan isolate (we designate as *FOP-RW2*) and *FOP-RW1* that was used by Buruchara and Camacho (1999), it is possible that the two Rwandan isolates that cause fusarium wilt are physiologically the same. It is also possible the Rwandan isolates (*FOP-RW1* and *FOP-RW2)* also belong to the same physiological race group as the southern American isolate used in the similar study by Ribeiro and Hagedorn (1979). The similarity is supported further by the fact that the 3 cultivars: G2333, G685 and Flora that were used in the current study were introduced from (southern) Latin America. This might suggest the co-evolution between the South American *F. oxysporum* f. sp. phaseoli pathogen and their local landraces, including the above introduced genotypes.

Conclusion and recommendation

These results indicate that the resistance against the Rwandan isolate *FOP-RW2* of *F. oxysporum* f. sp. phaseoli that causes fusarium wilt of climbing beans is conditioned by a major dominant gene (*Fop 1)* that is highly heritable. The improvement of susceptible commercial parents such as Umubano, G2333, for resistance against the disease can be achieved through backcross selection

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