International Scholars Journals

African Journal of Agriculture ISSN 2375-1134 Vol. 8 (1), pp. 001-008, May, 2021. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Rwandan susceptible common bean cultivars by introgression of pythium root rot resistance gene

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Accepted 22 May, 2021

A breeding scheme was carried out to introgress resistance genes to bean *Pythium* root rot in various commercial varieties grown in Rwanda. The achieved crosses were performed between three selected susceptible varieties (R617-97A, RWR 1668 and Urugezi) which are adapted to the various ecological production zones of Rwanda and two known sources of resistance to *Pythium* root rot (RWR719 and AND 1062). Following each inter varietal hybridization generation, a series of 4 successive back-crosses was achieved by using the susceptible parents as the initial parent lines to be improved for their respective behavior to *Pythium* root rot disease. At each back-cross generation, the PYAA 19_{800} SCAR marker linked to *Pythium* root rot resistance in the two sources of resistance (varieties RWR 719 and AND 1062) was used to identify and to proceed to early selection of progenies possessing the gene of interest. The target materials serving for the molecular analyses were prepared from young trifoliate leaves of 2-weeks bean plantlets. It was observed that at each back-cross generation, there were variable proportions of plants exhibiting presence of the resistance gene according to the SCAR marker profiles. In addition to that observation mainly based to molecular profile, it was also revealed that the proportion of bean seeds having the same color as the susceptible parent line was increasing progressively. Finally, to assess if the individual plants exhibiting the SCAR marker are effectively resistant to the *Pythium* root rot disease, inoculation tests were carried out with a *Pythium ultimum* strain on each of them. This ultimate biological evaluation revealed that all the plants showing the SCAR marker were resistant to development of *Pythium* root rot symptoms after inoculation, confirming thus real introgression of the resistance characteristics through the breeding scheme adopted in our work.

Key words: *Phaseolus, vulgaris*, *Pythium,* molecular, root rot, bean, genotype, resistance.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is the most important food legume crop grown worldwide (Wortmann and Allen, 1994; Wortmann et al., 1998; Buruchara, 2006). Beans are considered by many to be the perfect food as they are nutrient dense with high contents of protein, micronutrients, vitamins, dietary fiber, and also have a low glycemic index (wortmann and Allen, 1994; Bennink, 2005; Widers, 2006). Diverse forms of bean consumption including fresh or dry grains, green leaves and green pods (Kimani et al., 2006) are common in Rwanda. World annual global production of dry beans is estimated to reach 19.5 million t with Brazil being the highest producer with an estimated annual production of 4 million t (FAOSTAT, 2007). Production of common

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beans throughout the different production areas in the world is hampered by various biotic and abiotic factors leading to a continuous decline of the per unit production. In Rwanda where the bean production is estimated at a level 163,865 T (MINAGRI, 2009), the bean root rot disease has been found to constitute one of the major biotic constraints for production. Bean root rot caused by *Pythium* spp. is one of the most damaging diseases affecting common bean (*P. vulgaris*) in East and Central Africa sub-region where beans are grown in intensive agricultural production systems (Wortman et al., 1998).

A complete yield loss usually occurs when susceptible varieties are grown under environmental conditions which are favorable for the pathogen development like high level of humidity and low temperature varying between 14 and 17 (Buruchara and Rusuku, 1992). To reduce the level of production damages caused by his disease, several control strategies including the use of resistant varieties, performing soil amendments by using organic fertilizers, biological control based on a competition phenomenon between bean pathogenic isolates of *Pythium* and non pathogenic *Pythium* strains which do not cause any damage to bean plants (Spence, 2003). Moreover, the use of chemical applications through seed treatment has been shown to be efficient by ensuring a significant limitation of *Pythium* damages caused to beans (Bhardwaj et al., 1994). The use of resistant varieties is considered to be the most viable option for controlling *Pythium* bean root rot particularly for small-scale growers (Otsyula et al., 1998). Previous screen house and field evaluations carried out in Kenya, Rwanda and Uganda allowed identifying a few bean lines with resistance properties to *Pythium* root rot disease. Among those lines, the genotypes RWR 719 and AND1062 with resistance to that disease (Buruchara and Kimani, 1999) have been characterized as having a molecular marker associated to the resistance property.

Various investigations relative to inheritance of the bean resistance to *Pythium* in different varieties including RWR 719 and AND1062 is controlled by a single dominant gene (Otsyula et al., 2003). Similar observations were performed by Mahuku et al. (2007) on the line RWR 719. Once potential sources of resistance are identified, they can be used to ensure improvement of the resistance pattern of some popular commercial varieties. For that purpose, recurrent back-crossing following preliminary intervarietal hybridization is a traditional breeding method commonly employed to transfer alleles at one or more loci from a donor to an elite variety (Reyes-Valde´s, 2000). During the past two decades, the tendency to increase ability of transferring target genomic regions using molecular markers resulted in an important achievement of genetic mapping experiments aiming at developing molecular markers to be used for marker assisted back-crossing (MAB) (also called marker assisted selection (MAS), marker assisted introgression or molecular breeding). Molecular markers are tools that can be used as chromosome landmarks to facilitate assessing the effective introgression of chromosome segments (genes) associated with economically important traits (Semagn, 2006). That type of markers have been identified and/or used for MAB in several plant species, including maize, rice, wheat …and bean. Molecular markers do not require genetic engineering and cultivars to be developed by MAB, are not transgenic and therefore, do not face the public resistance against transgenic crops.

The success of MAB depends upon several factors like the distance between the closest markers and the target gene, the number of target genes to be transferred, the genetic base of the trait, the number of individuals that can be analyzed and the genetic background in which the target gene has to be transferred, the type of molecular marker(s) used, and available technical facilities (Francia et al., 2005). The most favorable case for MAB is when the molecular marker is located directly within the gene of interest (Dekkers, 2004). Several important genes in breeding for disease resistance and quality traits are inherited recessively. In conventional back-cross programs for introgression of a recessive target gene, that gene's presence or absence in a back-cross individual is determined by a phenotypic assay of progeny generated either by selfing or by crossing with the donor parent (Semagn et al., 2006). In the case of bean resistance to *Pythium* root rot , a SCAR marker named PYAA 19_{800} was characterized as being associated with *Pythium* root rot resistance gene in RWR 719 and AND 1062 (Mahuku, 2007). This marker was already validated and successfully used in selection for resistance to bean root rot. The use of MAB markers can constitute a very useful tool to speed up the breeding program as only individuals exhibiting the markers constitute the materials of interest to be analyzed in more details.

The objective of this work was to transfer the previously identified gene of resistance to *Pythium* species into susceptible commercial varieties adapted to the various ecological production zones of Rwanda. In that context, a breeding scheme based on a series of back-crosses between two resistant varieties (RWR 719 and AND1062) and the commercial susceptible varieties grown in Rwanda (R617-97A, RWR 1668 and Urugezi) was adopted.

MATERIALS AND METHODS

Genetic materials and study site

Seeds of three susceptible commercial cultivars were provided by the Rwanda national bean program (R617-97A, RWR 1668 and Urugezi), while seeds of the resistant varieties (RWR 719 and AND 1062) were provided by the CIAT Regional Office in Uganda. The genotype RWR 719 is a small seeded variety of Mesoamerican gene pool that is resistant to all species of *Pythium* while AND1062 is the only large seeded variety resistant to *Pythium* (Mukalazi et al., 2001). These cultivars were released in Rwanda based on various characteristics

Table 1. Type of reaction to *Pythium* and characteristics of genotypes used in the study.

Cultivars	Reaction to *Pythium*	Growth habit	Seed size	Seed color	Maturity (days)	Yield (ton/ha)	Altitude
Urugezi	Susceptible	Determinate	Medium	Red mottled	Medium	2	LA, MA
RWR 1668	Susceptible	Determinate	Large	Brown cream	Early	1.5 to 2	LA, MA, HA
R617-97A	Susceptible	Determinate	Large	White	Late	1.7 to 2.5	MA, HA
RWR 719	Resistant	Determinate	Small	Red	Late	0.9	LA, MA
AND 1062	Resistant	Determinate	Large	Kidney red	Medium		

LA: Low altitudes (900 to 400 m), MA: Middle altitudes (1400 to 1650 m), HA: High altitudes (1650 to 2300 m).

presented in Table 1. The experimental investigations were carried out at both laboratory and screen house levels in the CIAT regional centre of Kawanda-Uganda. This site is located at 0°25′05″ N and 32°31′54″ E at 1190 m above sea level (masl) with an average rainfall of 1224 mm per annum and average daily temperatures of 15.3°C (minimum) and 27.3°C (maximum).

Planting conditions

The recipient cultivars R617-17A, RWR 1668 and Urugezi were grown alongside the donor parent RWR 719 and AND 1062 in the screen house. The loam soil mixed with sand and organic manure (in ratios of 3:1:1) was sterilized by steam sterilization. Plastic pots (3 kg) were filled at 3/4 with soil and three seeds were sewed in each pot. The so treated pots were watered whenever there was a need. NPK fertilizer was applied at flowering stage to improve plant vigor. For the crossing block establishment, recipient as well as donor parents were planted at different times (3 to 4 days interval) to ensure that there were constant flowers for both the donor and recipient plants.

Hybridization protocol

The female recipient plants were crossed with the male donor plants to develop an F_1 population. For back-crossing population development, F_1 plants were used as females while the recurrent parents were used as male parents. Male flowers were collected using tweezers when they were just opened. Flowers were collected the day of pollination and kept in paper bags for the shortest possible time until they were used. Buds selected on the female plants had increased in size and had lost the green color of immature buds but had not yet started to split. Such buds were considered as not yet self-pollinated and were ready to open a day later. Female flowers were opened and anthers were carefully removed to reduce or avoid self-pollination to take place since the bean flower was complete and therefore capable of self-pollination.

To realize pollination, the stigma of male flower coated with pollen was removed using tweezers. The stigma of female flower was exposed and dusted with pollen from the male plant. The crossing was done early in the morning or later in the evening to avoid any damage which would be caused by the sun heat. Instruments used were washed thoroughly in alcohol to eliminate any pollen grain from previous flowers. The pollinated flower was then closed back with its petals to reduce possible natural crossing and also to preserve humidity around the stigma. Fertilized flowers were marked with a tag (Oscar and Luz, 1987) with primary information concerning the cross for easy further management of the crossing program. In the present work, the breeding scheme included a F1 generation and a series of 4 back-crossing generations as presented in the Figure 3.

Leaf samples' preparation and DNA extraction

Total genomic DNA was extracted from young trifoliate leaves collected from 2-week-old plants in the screen house. The leaves were plucked from the plants and put in plastic bags labeled with the right identification number. The bags were then put on ice and transferred to the laboratory for DNA extraction using the FTA card procedure and subsequent analysis by the polymerase chain reaction using molecular marker ($PYAA19_{800}$). The collected leaves were spotted on the FTA plant saver cards following Whatman technologies. The samples were overlaid with parafilm and crushed using a porcelain pestle and mortar, followed by the following successive steps:

i) The crushed leaf tissue was left to dry at room temperature for 1 h;
ii) A 2 mm leaf disc was excised using a Harris unicore borer;
iii) The disc was washed two times using FTA purification reagent (100 µl);
iv) The material was incubated for 3 min at room temperature;
v) The disc was also washed again two times by using isopropanol (100 µl);
vi) The material was incubated for 3 min at room temperature;
vii) The disc was dried in PCR tube;
viii) Sample discs were thus ready for addition of PCR master mix.

Polymerase chain reaction analyses

The PCR master mix consisted of 0.2 mM of dNTPs, 2 mM $MgCl_2$, 1 µ/25 µl of Taq polymerase,1X PCR Buffer and 0.4 µM of each primer. Sequences of the used primers were 5' -TTA GGC ATG TTA ATT CAC GTT GG-3' for primer 1 and 5'- TGA GGC GTG TAA GGT CAG AG- 3' for primer 2 (Mahuku, 2007). The 25 µl-reaction PCR reaction volume was subjected to 34 amplification cycles in a BIO RAD MyCycler thermal cycler consisting of 1 cycle 94°C for 5 min, and 34 cycles including each the steps of denaturation at 94°C for 40 s, annealing at 63°C for 40 s, and extension at 72°C for 40 seconds. These cycles were followed by a final extension for 7 min at 72°C and a holding temperature of 4°C. Amplification products were separated through electrophoresis migration in a 1.2% agarose gel covered by a 0.5X TBE buffer under a voltage of 100 V for 45 min. For the visualization, the gel containing ethidium bromide (0.5/ml) was lighted with ultraviolet light and photographed for scoring (Mahuku, 2007).

Pathogenicity tests on the back-cross progenies

Inoculum of one *P. ultimum* strain was multiplied by plating mycelia on autoclaved millet grains (100 g) mixed with 200 ml of water in

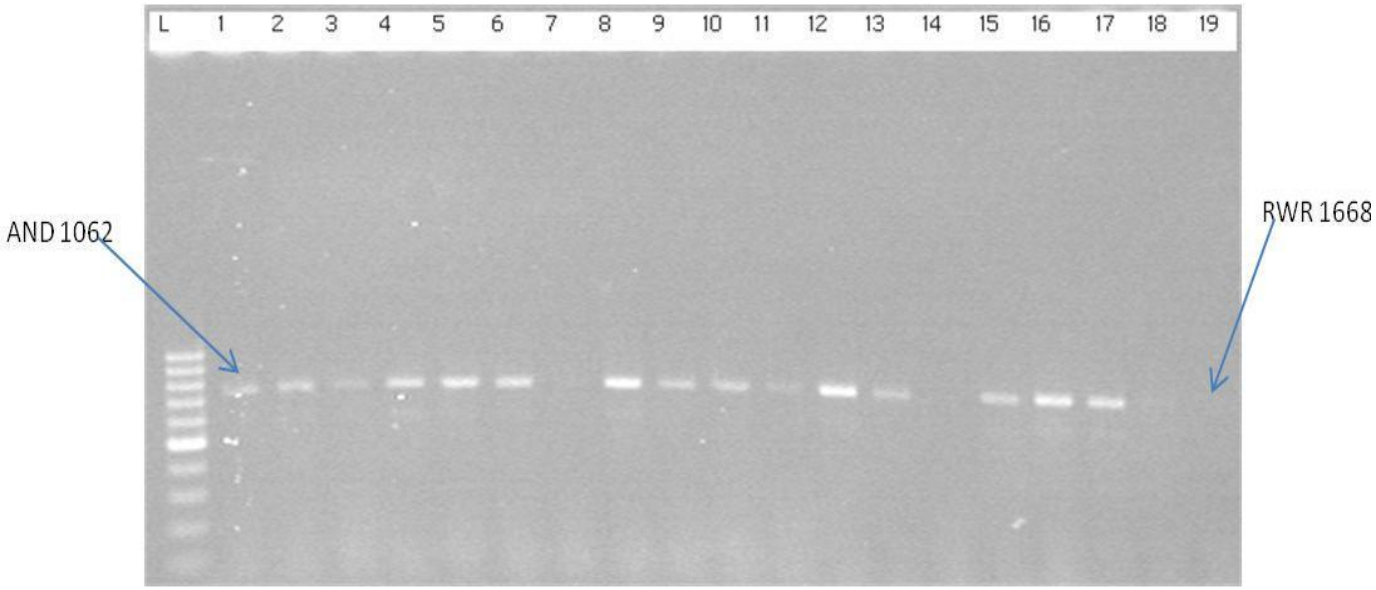

Figure 1. Amplification of BC_4 plants samples from [(BC_4 RWR 1668 X AND 1062) X RWR 1668] cross. L= DNA ladder.

500 ml bottles. After two weeks of incubation under darkness and 25°C, pre sterilized soil was mixed with the infested millet at a ratio of 1:10 v/v in wooden trays of 42 x 72 cm. Each tray contained 10 plants of each bean cultivar used in this evaluation analysis. The trays were set up in a completely randomized block design (CRBD) with three replications. After germination, the seedlings were watered two times per day to provide a favorable environment for the pathogen establishment and development. Three weeks after emergence of the seedlings, the surviving plants were uprooted and washed with water to remove soil. Severity of root rot symptoms was then assessed using the CIAT visual scale whose scores vary from 1 to 9 (Abawi and Pastor- Corrales, 1990).

Cultivars that had an average disease score of 1 to 2 were considered as being resistant while those with an average score of 3 to 5 were considered tolerant and those with an average score of 6 to 9 were considered to be susceptible (Abawi and Pastor-Corrales, 1990).

RESULTS

Genotypic results

All the individual plants of the F_1 generation were supposed to be uniform and heterozygous for the targeted resistance characteristics. Based on the morphological aspect of either seeds as well as of their resulting plantlets, the uniformity within F1 generation was confirmed (Figure 1). Moreover, whatever the hybridization scheme, all the seeds of the F1 generation were of the same morphological aspect as their female parent line. The F1 plants were back-crossed as female parents to the recurrent parents. At the first back-cross (BC_1), progenies were analyzed genotypically for presence of the gene of resistance to *Pythium*. At the different back-cross generations, molecular analyses using the PCR technology were undertaken to assess the presence of the targeted gene conferring resistance to *Pythium* root rot disease. Globally, it was observed that the molecular pattern was variable at the level of each back-cross generation as there was a mixture of individuals showing the targeted marker and other individuals without the marker of interest in this study (Figure 1). The evolution of proportion of individuals showing the presence of the targeted zone is showed in the Table 2 using the $PYA19_{800}$ SCAR marker.

The results based on the different proportions of individuals with the different molecular patterns allowed performing chi square analysis for each type of hybridization and that at each back-cross generation. The back-cross populations BC1 (RWR 1668 x AND 1062) x RWR 1668 and (Urugezi x RWR 719) x Urugezi did not fit the 1:1 and 1:3 ratios at P = 0.05. However, the BC1 ((RWR 1668 x RWR 719) x RWR 1668) was not significant at 1:1, but significant at 1:3, while, BC1 ((RWR 617-97 x RWR 719) x RWR 617-97), BC1 ((Urugezi x AND 1062) x Urugezi) and ((RWR 617-97 x AND 1062) x RWR 617-97) fitted both ratios at P=0.05 (Table 2). The resistant progenies were crossed to the recurrent parents to generate BC_2 progenies.

The BC2 populations were analyzed as for the BC1 and results (Table 2) showed that χ^2 goodness-of-fit to the 1:1 and 1:3 ratios of the cross code ((RWR 1668 x RWR 719) x RWR 1668), ((RWR 1668 x AND 1062) x RWR 1668), ((Urugezi x AND 1062) x Urugezi) and [(R 617-97 x RWR 719) x RWR 617-97) were highly signi-ficant for both ratios. ((Urugezi x RWR 719) x Urugezi) was significant at 1:1 and highly significant at 1:3 ratios. Only ((R 617-97 x AND 1062) x RWR 617-97) was not significant at

Table 2. Test of χ^2 for a goodness-of-fit to the 1:1 and 1:3 ratio of different cross code of BC_1, BC_2, BC_3 and BC_4.

Crosses	No. Plants		χ^2 BC1		No. plants		χ^2 BC2		No. plants		χ^2 BC3		No. plants		χ^2 BC4	
	R	S	1:1	1:3	R	S	1:1	1:3	R	S	1:1	1:3	R	S	1:1	1:3
(RWR 1668 x RWR 719) x RWR 1668	6	14	ns	4.83 *	5	35	21,03 **	20.68 **	18	22	ns	6.28*	13	27	ns	9.28**
(RWR 1668 x AND 1062) x RWR 1668	11	9	ns	Ns	10	30	9.03 **	12.68 **	24	16	ns	7.08**	11	29	**	11.42**
(URUGEZI x RWR 719) x URUGEZI	11	9	ns	Ns	12	28	5.63 *	10.28 **	19	21	ns	6.08*	19	21	ns	6.08*
(URUGEZI x AND 1062) x URUGEZI	4	16	6.05 *	7.37 **	7	33	15.63 **	17.08 **	14	26	ns	8.42**	26	14	ns	8.42**
(R 617-97 x RWR 719) x RWR 617-97	3	17	8.45 **	8.97 **	6	34	18.23 **	18.82 **	15	25	ns	7.68**	16	24	ns	7.08**
(R 617-97 x AND 1062) x RWR 617-97	4	16	6.05	7.37**	17	23	ns	6.62 *	10	30	9.03**	12.68**	16	24	ns	7.08**

Progeny size analyzed: n = 20 for BC_1 and n = 40 for BC_2, BC_3 and BC_4. R: resistant, S: susceptible, ns: not significant, *, **: significant at 5 and 1% probability level, respectively.

1:1 ratio but significant at 1:3 ratio. This cross code had the highest number of resis-tant progenies (Table 2), followed by ((Urugezi x RWR 719) x Urugezi). With the BC_3 results, it appeared that only one cross code ((R 617-97 x AND 1062) x RWR 617-97)) fitted the 1:1 ratio at P=0.01 while all the other crosses did not generate results estimated to be significant when analyzed for fitting that 1:1 ratio. At the BC_4 generation results, only one cross code ((RWR 1668 x (RWR 1668 x AND 1062)) fitted the 1:1 ratio at P = 0.01 while the all the other crosses were not significant. This cross code had the lowest number of plants showing presence of the marker of interest compared to the other crosses (Table 2).

However all the cross codes were highly signifi-cant at the 1:3 ratio (Table 2) apart from the cross code ((URUGEZI x (URUGEZI x RWR 719)) which was only significant at the 1:3 ratio. Almost 42% of the analyzed plants at the BC4 generation showed presence of the marker of interest based on the molecular analyses. At this same BC4 generation were used to perform a pathogenicity test carried out in a screen house. From the aforementioned results, there is evidence of a progressive increase of the number of individual plants showing the presence of the marker PYAA 19_{800} at each successive back-cross level. On the other side, it appeared that the color of seed started to be fixed for most of the progenies at the BC4 generation (Figure 2).

Pathogenicity tests

Results of the pathogenicity tests are presented in the Table 3 where the data were collected and expressed as scores of the disease severity. As shown in the table, two references were used in the inoculation tests; the susceptible reference was the commercial variety CAL 96 for which the observed symptoms were scored at an average disease severity of 8.47 while the resistant reference was the variety RWR 719 with an average of disease severity established at 1.33. All the plants resulting from the breeding scheme carried out in our study showed a level of root rot symptoms varying between the two reference varieties. In fact, the disease severity varied between 2.23 and 3.73 for the plants resulting from the breeding scheme.

According to the adopted scoring system, all these plants were estimated to be resistant except progenies of the crosses URUGEZI X RWR 719 and R 617-97A X RWR 719 which were scored as tolerant.

DISCUSSION

In the present study, it was undertaken to evaluate the feasibility of improving the resistance level to *Pythium* root rot disease in different common bean varieties used in Rwanda. The work was carried out by performing a series of intervarietal hybridizations followed by back-crosses using the parent lines to be improved as the male parent. To speed up the selection process, a molecular marker named PYAA198_{00} (Mahuku, 2007) was used for an early identifi-cation of individuals holding the characteristics of resistance to *Pythium* root rot disease. The patho-genicity tests based on biological tests involving inoculation assays constituted the ultimate

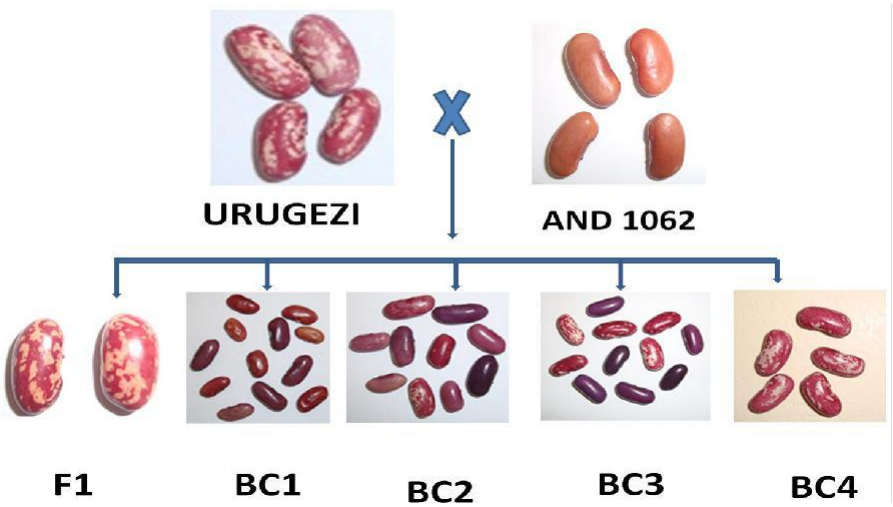

Figure 2. Evolution of seed color at the different generations of the breeding scheme: case of the crosses relative to URUGEZI X AND 1062.

Crossing	Type of crossing
Original cross	Cultivar A rr x Resistant RR
1 st Backcross	F_1 Rr x Cultivar A rr 50% genes from A
2 nd Backcross	BC_1 Rr:rr x Cultivar A rr 75% genes from A
3 rd Backcross	BC2 Rr:rr X Cultivar A rr 87,5% genes from A
4 th Backcross	BC3 Rr:rr X Cultivar A rr 93,75% genes from A
	BC4 Rr:rr 96,875% genes from A
	Self Rr plants from BC4 to obtain plants homozygous for RR
	1RR:2Rr :1rr

Figure 3. Scheme of crossing between susceptible and resistant varieties to *Pythium* spp. Source: (Poelman and Sleper, 1995).

Table 3. Expression of disease severity of progenies from back-cross4 progenies.

Variety	Mean	t Grouping	N	Disease expression of bean cultivars
CAL 96	8.47	A	30	Susceptible
URUGEZI X RWR 719	3.73	B	30	Tolerant
R 617-97A X RWR 719	3.20	BC	30	Tolerant
R 617-97A X AND 1062	2.90	DC	30	Resistant
URUGEZI X AND 1062	2.73	DC	30	Resistant
RWR 1668 X AND 1062	2.67	DC	30	Resistant
RWR 1668 X RWR 719	2.23	D	30	Resistant
RWR 719	1.33	E	30	Resistant

Means with the same letter are not significantly different, α= 0.05, LSD= 0.76, SE=0.27, Pr> |t|<.0001. N= Number of plant evaluated per bean cultivar.

assessment of the effective introgression of the resistance properties. It was confirmed that the disease severity of symptoms recorded on individual plants issued from the breeding protocol was lower than the one recorded in susceptible varieties. This observation allowed classifying these plant materials resulting from the breeding process in the category of tolerant or resistant genotypes whatever the crosses involved. Given that profile of resistance to *Pythium* found in the progenies from back-crosses between (RWR 1668, R 617-97A and Urugezi, as recipient parents) and the selected sources of resistance (RWR 719 and AND 1062, as donor parents), it can be concluded that the introgression of the resistance properties was successful.

In these conditions, the used donor parents can be proposed to be considered as efficient sources of resistance in breeding programs aiming to increase the resistance to *Pythium* root rot and thus to facilitate field control of the disease. Other authors have already proved the inheritance of resistance properties in common bean by using similar breeding scheme to ensure improvement disease control. That was the case in the study of inheritance and transfer of root rot (*Pythium*) resistance to bean varieties (Otsyula et al., 2003). On the other side, the breeding scheme (Poelman and Sleper, 1995) used in the present investigation was also highly efficient. Based on those facts, the 2 donor lines as well as the breeding protocol used in this work could be used to perform improvement of common bean resistance to *Pythium* bean root rot in Rwanda.

In a similar study carried out by Otsyula et al. (2003) the genetic resistance found in the two varieties (RWR 719 and AND 1062) has been proved to be effective against all the *Pythium* species collected from Rwanda. Observations performed through our study in terms of inheritance of the resistance properties were in accordance with those revealed by Buruchara et al. (1999) and Otsyula et al. (2003). In fact, these authors showed that resistance to *Pythium* root rot in the variety RWR 719 was controlled by a dominant and simply inherited gene. With our results, it is further demonstrated that genes of interest in populations developed from the small seeded RWR 719 genotype and the large seeded AND 1062 genotype exhibit resistance pattern to *Pythium* root rot disease and give goodness of fit for segregating population in back-crosses. Based on the generated data and the results previously produced by other authors (Buruchara and Kimani, 1999; Buruchara and Mayanja, 2001) it was concluded that the genetic basis for inheritance of resistance to *Pythium* root rot in common beans is expressed in the similar fashion regardless of the source. Moreover, there is no relationship as the resistance behavior was the same even if the source is of Mesoamerican or Andean gene pool genotype.

Our results revealed that a series of six new genotypes showing resistance or tolerance to Pythium root rot disease were obtained by at the end of the adopted breeding process while initially all the tested Rwandan varieties were susceptible. Apart from the number of progenies with the resistance properties to *Pythium*, it was possible to observe that the color of seed started to be fixed for all the progenies at the BC 4 step. Molecular analyses performed with the BC4 progenies facilitated identification of progenies holding the resistance marker revealing the presence of resistance gene. These progenies of interest have thus been involved in of the ultimate pathogenicity tests carried out in screen house. Development of root rot symptoms following the pathogenicity tests confirmed the resistance profile in these progenies issuing from the back-cross program.

In conclusion, control of *Pythium* root rot disease by using genetic resistance would be easily achievable as once commercial and popular varieties are available, they can easily be improved by adopting a simple protocol of crossing with the already identified sources of resistance.

ACKNOWLEDGMENT

The authors would like to express their warmest thanks to

CIAT for having given technical assistance and laboratory facilities to Mr. Nzungize during his investigations. Moreover, the project was supported by the Belgian Technical Cooperation, the Kirkhouse Trust and the Rwanda Agricultural Research Institute which are thanked through this work.

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