A gene that confers resistance to Pythium root rot in common bean: Genetic characterization and development of molecualr markers.

GEORGE MAHUKU[1], R. Buruchara[2], M. Navia[1], and R. Otsyula[3]

[1]Centro Internacional de Agricultura Tropical, A. A. 6713, Colombia, South America;
[2]Centro Internacional de Agricultura Tropical, Pan Africa Bean Research Alliance, P.O. Box 6247, Kampala, Uganda;
[3]Kenya Agricultural Research Institute, P.O. Box 169, Kakamega, Kenya

Introduction

Bean root rot, caused by several *Pythium* species is one of the most destructive diseases affecting common bean (*Phaseolus vulgaris*) in East and Central Africa where beans are grown in intensive agricultural production systems (Wortman et al., 1998). Complete yield loss usually occurs when susceptible varieties are used and environmental conditions are favorable for pathogen development (Buruchara and Rusuku 1992; Otsyula et al., 1998). The fungus infects the root and lower stem parts of the plant, and depending on the time of infection and prevailing environmental conditions, symptoms may be manifested as seed rot, damping-off, root rot, foliar blight or pod rot (Abawi and Pastor-Corrales, 1990) (Figure 1).

Use of resistant cultivars is considered to be the most viable option for controlling bean root rot particularly for small-scale growers (Otsyula et al., 1998). To curb the effects of this disease, identification and transfer of resistance to bean genotypes preferred by resource poor small holder farmers is an important consideration. A few bean genotypes with resistance to pythium root rot have been identified, among them RWR 719 (Buruchara and Rusuku 1992). Field and screen house evaluations of RWR 719 using several *Pythium* species confirmed its potential as a source of resistance to this pathogen, although information on the genetics of resistance is needed to effectively utilize its resistance. This study was initiated to define the pattern of inheritance of pythium resistance in RWR 719, and to develop molecular markers that are tightly linked to the resistance gene(s) so as to facilitate the introgression of resistance into varieties preferred by small-scale farmers in east and central Africa

Figure 1: Pythium root rot symptoms under field conditions (Kabale, western Uganda)

Objective

To elucidate the genetics of pythium root rot resistance and identify molecular markers linked to the resistance genes in the cultivar RWR 719.

Materials and methods

Plant material: The resistant variety RWR 719 was crossed to the susceptible commercial cultivar, GLP2 to establish F_1, F_2, and backcross populations to susceptible (BC_S) and resistant progenitors (BC_R).

Fungal Isolate and inoculum production: Throughout this study, *Pythium ultimum*, previously established as the most important and widely distributed species causing bean root rots in East and Central Africa (Mukalazi et al, 2001), was used. Inoculum production, and inoculation were done as described previously (Mukalasi et al, 2001). Seeds of parental materials (RWR 719 and GLP 2), F_1, F_2, BC_S and BC_R were planted in inoculated soil in wooden trays. Germinated seedlings were watered 2 times a day for three weeks to provide a favorable environment for fungal infection, establishment and development. Individual seedlings were uprooted, washed in tap water and roots scored using the CIAT 1-9 scale (van Schoonhoven and Pastor-Corrales, 1987). Plants with no or limited symptoms (score 1-3) were rated as resistant, and the rest of the plants as susceptible. The class frequencies obtained were tested for goodness of fit to theoretical ratios using the chi-square tests.

DNA extraction and Marker identification: Young trifoliate leaves were collected from the two parents, and from resistant and susceptible F_2 progenies, and DNA was extracted using the procedure described by Mahuku (2004). Five resistant and 5 susceptible plants, including the parents were used to evaluate 300 RAPD and 50 RAMS primers as previously described (CIAT, 2004). Candidate markers showing evidence of correlation to disease resistance or susceptibility were further evaluated on an additional 10 resistant and susceptible F_2 plants. Where polymorphism was maintained, the potential markers were evaluated on the entire F_2 population (Table 1). The marker scoring data in the F_2 were merged with the disease scoring data for linkage analysis using the computer program MAPMAKER (Lander et al., 1987).

Table 1. Segregation ratios for resistance to *Pythium ultimum* var. ultimum in a GLP 2 x RWR 719 cross

Pedigree	Generation	Progenies Resistant	Segregating	Susceptible	Expected ratio	X^2	P
GLP 2	P1	0		60			
RWR 719	P2	60		0			
GLP 2 x RWR 719	F_1	60		0	1:0	0.00	1.0
GLP 2 x RWR 719	F_2	216		83	3:1	1.30	20- 30
F_1 x GLP 2	BC_S	49		40	1:1	0.96	30- 50
F_1 x RWR 719	BC_R	88		0	1:0	0.00	1.0
Populations	$F_{2:3}$ families	49	88	41	1:2:1	2.99	20- 30

Establishing the suitability of candidate marker for MAS: Candidate markers were evaluated on nine bean genotypes (RWR 719, MLB 49-89A, AND 1062, A 240, SCAM 80-CM/15, MEX 54, CAL 96, Urugezi and GLP 2), that are either resistant or susceptible to pythium root rots under greenhouse and field evaluations (Table 2).

Table 2: Response of common bean genotypes to inoculation with three-root rot causing *Pythium* species.

Entry	*Pythium* species		
	(*P. ultimum* var. *ultimum*)	(*P. sulcogenisporium*)	(*P. chamaehyphon*)
MLB-49-89A	1	1	1
MLB-40-89A	1	1	1
RWR 719	1	1	1
Scam80-cm 15	1	1	1
AND 1064	1	1	-
AND 1062	1	1	1
KWR 1091	1	1	1
GLP 585	9	9	9
Scam- KWD	9	9	9
GLP2	9	9	9
URUGEZI	9	9	9
CAL 96	9	9	9

Development of SCAR markers: Candidate fragments were excised from agarose gels, cloned and sequenced as described by Mahuku et al. (2004). Primers were designed using the Primer3 software (Center for Genome Research, Whitehead Institute, MA, USA - http://www-genome.wi.mit.edu/cgi-bin/primer/primer3). Developed primers were used to amplify DNA from parental materials, and two resistant and susceptible F_2 individuals. If polymorphism was maintained, the designed SCAR primers were tested in ten resistant and ten susceptible individuals. In the case of an identical sequence length, the fragment from the susceptible individuals was cloned and sequenced. The sequences derived from resistant and susceptible individuals were then aligned using the program MEGALIGN within DNAStar, and where possible the primer pairs were re-designed to exploit differences between the resistant and susceptible sequences.

Results and discussion

Nature and inheritance of Pythium resistance in RWR 719: All F_1 plants were resistant to *P. ultimum*, suggesting that a dominant gene(s) conditioned root rot resistance (Table 1). Segregation for resistance in the F_2 population was consistent with a 3:1 ratio (X2= 1.309), while that for the backcross to susceptible parent (GLP2) and resistant parent (RWR719) fit a 1:1 ratio (X2=0.962), and 1:0 ratio (X2=0.000) respectively, and a 1:2:1 in the $F_{2:3}$ population (Table 1). These results support the hypothesis that a single dominant gene in RWR 719 controls resistance to *P. ultimum* var. *ultimum*.

Marker identification: Of the 300 RAPD and 50 RAMS primers evaluated, two RAPD primers, (OPAA19 and OPBA08) and one RAMS marker (VHVGT)5G) segregated in coupling phase with the resistance gene in RWR 719 (Figure 2 A, B, and C). Linkage analysis after testing the marker on 150 F2 individual plants showed that the OPAA19 marker was located at 1.5 cM, the OPBA08 at 4 .0 cM and (GT)n primer at 6.3 cM from the resistance gene.

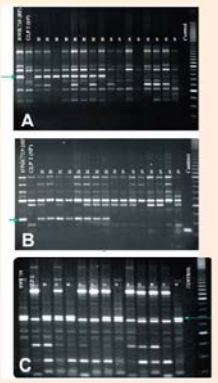

Figure 2: RAPD (A) OPAA19, (B)OPBA08, and RAMS GT (VHVGT)5G) linked in coupling to the pythium resistant gene in the common bean genotype, RWR 719.

Validation of markers for MAS: Amplification of parental materials and selected susceptible and resistant bean genotypes revealed the potential of all these markers for marker assisted selection (MAS) breeding. The fragments associated with resistance were present in all resistant and absent from susceptible genotypes (Figure 3A and B), raising the possibility that the resistant genotypes tested in this study carry the same resistance gene locus, with the same or different alleles for conditioning pythium root rot resistance. In addition, all the bred lines tested (RWR 719, AND1062, SCAM 80/15 and MLB49-89A) have A240 in their pedigree. Greenhouse evaluations of A240 showed that it was resistant to *P. ultimum* var. *ultimum*, and the fragment associated with resistance for all potential markers was present in A240. It is likely that this genotype is the origin of resistance to pythium root rots. We are currently evaluating genotypes other genotypes that have A240 as one of the parents to test this hypothesis.

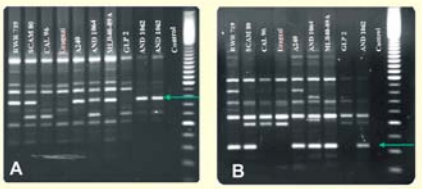

Figure 3: Validation of molecular markers outside the mapping population: (A) OPAA19 and (B) OPBA08. The fragments associated with resistance are present in all resistant and absent from susceptible genotypes.

SCAR Marker: Of the three SCAR markers developed, only the OPAA19 derived SCAR marker was polymorphic and co-dominant (Figure 4). The SCAR primers derived from BA08 and (GT)n amplified a similar sized fragment from susceptible and resistant plants. PCR products from resistant and susceptible parents amplified using these primers were cloned and sequenced. Alignment of resistant and susceptible plant sequences revealed polymporphisms and new primers targeting these differences were designed and are currently being synthesized. In addition, RILs have been developed from the RWR 719 x GLP 2 cross and these will aid further identification and development of other markers, and increase the efficiency of marker use in MAS.

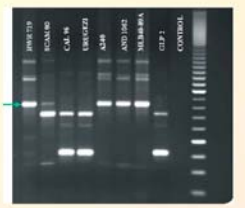

Figure 4: Amplification of resistant and susceptible bean genotypes using the SCAR primers derived from the OPAA19. The fragment associated with resistance was present in resistant and absent from susceptible genotypes.

Conclusions

- Resistance of RWR 719 to *P. ultimum* var. *ultimum* is simply inherited and controlled by single dominant gene. There is a need for allelism test to establish the independence of pythium resistance gene in RWR 719 to those in other bean genotypes, e. g. AND1062, A240, SCAM 80/15 and MLB49-89A.
- Three markers linked in coupling to the resistance gene in RWR 719 were identified, and the potential of these markers in MAS was established.
- A SCAR marker derived from the OPAA19 RAPD primer was developed and its potential utility for MAS demonstrated. We are in the process of validating this SCAR marker outside the mapping population. In addition, we are currently in the process of developing SCAR markers for the OPBA08 and VHVGT)5G markers.

References

1. Abawi, G.S. and Pastor-Corrales, M.A.; (1990). Root rots of bean in Latin America and Africa: diagnosis, research, methodologies and management strategies. CIAT, Cali, Colombia, 114 pp
2. Buruchara, R.A. and Rusuku, G. (1992). Root rots in the Great Lakes Region. Proc. of the Pan-African Bean Pathology Working Group Meeting, Thika, Kenya. May 26-30, 1992. CIAT Workshop Series No. 23. pp.49-55.
3. Mahuku, G. S. 2004. A simple extraction method suitable for PCR-based analysis of plant, fungal, and bacterial DNA. Plant Molecular Biology Reporter 22: 71-81
4. Mahuku, G., Montoya, C., Henríquez, M.A., Jara, C., Teran, H., and Beebe, S. 2004. Inheritance and Characterization of the Angular Leaf Spot Resistance Gene in the Common Bean Accession, G 10474 and Identification of an AFLP Marker Linked to the Resistance Gene. Crop Science 44: 1817-1824.
5. Mukalazi J. R. Buruchara, J. Carder, S. Muthumeenakshi, E. Adipala, F. Opio G. White, T. Pettitt and N.J. Spence (2001). Characterization of *Pythium* spp. Pathogenic to common beans in Uganga. African Crop Sci. Conference Lagos, Nigeria.
6. Otsyula, R.M.; S.I. Ajanga; R.A. Buruchara and C.S. Wortmann (1998). Development of an integrated bean root rot control strategy for western Kenya. African Crops Science Journal 6:61-67.

Wortmann, C. S., Kirkby, R. A., Eledu, C. A., and Allen, D. J. 1998. Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa. CIAT, Cali, Colombia.