Second RUFORUM Biennial Meeting 20 - 24 September 2010, Entebbe, Uganda

Research Application Summary

Assessing the efficacy of pyramided genes in conferring dual and durable resistance to bean anthracnose and root rot

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Abstract

Bean anthracnose (*Colletotricum lindemuthianum*) and root rots are important diseases of beans in Uganda. The co-existence of both diseases on the crop in farmers' fields and the ability of these pathogens to overcome incorporated resistances in commercial varieties have greatly undermined previous breeding efforts leading to severe yield losses in bean varieties that had been previously released with single gene resistance. This has created the need for pyramiding genes to broaden resistance against anthracnose and root rot diseases. The breeding period could be considerably reduced by the use of molecular markers which help to track the pyramided genes concurrently. Therefore, this study aims at developing bean genotypes with dual and broader resistance to bean anthracnose and bean root rots through a gene pyramiding programme aided by the use of SCAR markers for quick selection and gene tracking.

Key words: *Fusarium* spp., marker assisted selection, multiple resistance, *Pythium* spp., SCAR

Résumé

L'anthracnose de haricot (*Colletotricum lindemuthianum*) et le pourrissement des racines sont d'importantes maladies du haricot en Ouganda. La co-existence de deux maladies sur les cultures dans les champs des agriculteurs et la capacité de ces agents pathogènes pour surmonter les résistances incorporées dans les variétés commerciales ont considérablement miné les efforts de sélection précédente conduisant à des pertes de rendement importantes dans les variétés de haricots qui avaient déjà été libérées avec un seul gène de résistance. Cela a créé la nécessité de pyramidage des gènes pour élargir la résistance contre les maladies d'anthracnose et de putréfaction des racines. La période de reproduction pourrait être considérablement réduite par l'utilisation de marqueurs moléculaires qui permettent

de suivre les gènes cumulés en même temps. Par conséquent, cette étude vise à développer des génotypes de haricots avec une double et large résistance à l'anthracnose du haricot et à la pourrissement des racines de haricot par un programme de pyramidage des gènes, aidé par l'utilisation de marqueurs SCAR pour une sélection rapide et le suivi des gènes.

Mots clés: Espèces de *Fusarium*, sélection assistée par marqueurs, résistance multiple, espèces de *Pythium*, SCAR

Background

Colletotrichum lindemuthianum the cause of beans anthracnose disease possesses a high degree of genetic variability. It is thus implicated that single gene resistance alone is not adequate to offer effective and durable resistance aginst anthracnose in common bean varieties. The few available resistant varieties have poor marketability, while the available market-class cultivars are all susceptible to anthracnose. On the other hand, root rot is an emerging and increasing problem associated with beans growing on nutrient depleted soils and occurs widely in Uganda resulting in almost 100% yield losses in susceptible varieties (Mukankusi, 2007). The most important root rots in Uganda are caused by the fungi, *Pythium* spp., *Rhizoctonia* spp. and *Fusarium* spp. There is also limited number of potential sources of resistance against root rot, particularly *Pythium* root rot.

Both diseases co-exist on the crop in farmers' fields and the pathogens have the ability to overcome incorporated resistances. This has greatly undermined previous breeding efforts for varieties with single gene resistance. These diseaes need to be controlled, otherwise they will undermine the potential of beans as a food security crop, source of income and as a main source of dietary protein for the majority of Ugandans.

This study therefore aims at developing bean genotypes with dual and durable resistance to bean anthracnose and bean root rots through a gene pyramiding program aided by the use of SCAR markers for quick selection and gene tracking. The effectiveness of single genes, pyramided genes and different pyramided gene combinations against different races of anthracnose and different root rot pathogens will be evaluated.

Literature Summary

Bean anthracnose, caused by *Colletotrichum lindemuthianum*, an imperfect, anamorphic fungus is one of the most widespread and economically important fungal diseases of common bean in

Uganda (Nkalubo, 2006). High variability for this pathogen has been reported in the center of origin of common bean. This is also true in Uganda where different pathogenic races have been confirmed (Nkalubo, 2006). In addition to anthracnose, the incidence of bean root rot in Uganda is increasing as the pressure on the land increases. Root rot is caused by a complex of fungal pathogens, resident in the soil. These include species of *Pythium*, *Fusarium solani* f. sp *phaseoli*, *Rhizoctonia solani* and *Sclerotium rolfsii*. Of these *Pythium* and *Fusarium* species have been reported to be the most improtant, on the basis of spatial distribution, damage and effect on yield, causing complete crop failures in some seasons (CIAT, 2003).

Use of resistant, adapted and acceptable cultivars is an effective management option for disease control particularly for small-scale farmers. Resistance mechanisms and genes have been identified in the common bean for both anthracnose and root rot (Otsyula *et al*., 2005). Pyramiding these genes for resistance may be an effective strategy for controlling pathogens that pose a moderate risk of evolving virulent pathotypes. Gene pyramiding has been successfully applied in several crop breeding programs, and many varieties and lines possessing multiple attributes have been produced. Pyramiding resistance genes using molecular markers has been proposed as a more efficient selection method for disease-resistant beans (Garzon *et al*., 2008).

Study Description

Seven parental materials will be used in the following gene pyramiding scheme; (((G2333 x PI207262) x RWR719) x K132, NABE4, NABE13 & NABE14). The parents G2333 and PI207262 are donors for anthracnose resistance genes, parent RWR719 is a donor for *Pythium* root rot resistance gene, while the parents K132, NABE4, NABE13 and NABE 14 are the susceptible market class varieties to be improved. DNA extraction and SCAR marker screening and assisted selection will be carried out at different generations in order to track the inherited resistance genes among the segregating populations at different generations. Sample DNA will be amplified using the standard Polymerase Chain Reaction (PCR) protocol with minor modifications. The PCR products will be separated on 6% non-denaturing polyacrylamide gel and will be visualized under Ultra Violet (UV) light following staining with ethidium bromide.

To evaluate the effectiveness of the single genes, pyramided genes and gene combinations against different anthracnose

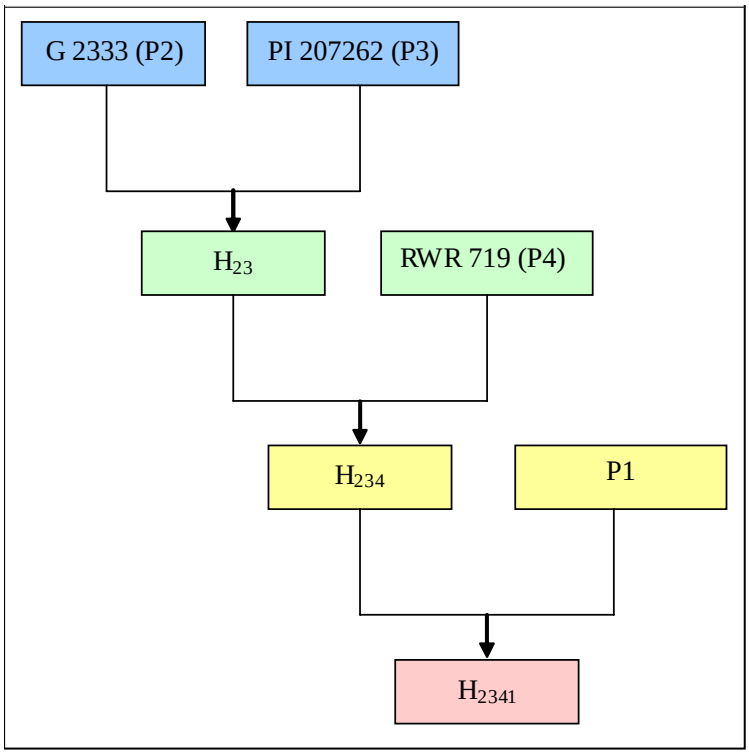

Figure 1. Pyramiding scheme used for crossing. Parents G2333 and PI207262 are the donors for anthracnose resistance; RWR719 is the donor for Pythium resistance; and P1 are the susceptible lines K132, NABE 4, NABE 13 & NABE 14 to be improved.

isolates and root rot pathogens, the F_2 and F_3 plants of the above crosses will be inoculated with the two pathogens. Inoculum will be raised following standard procedures for the two pathogens. Disease severity will be scored and data used to calculate AUDPCs and later subjected to ANOVA.

Research Application

Information valuable to bean breeders will be generated about the effectiveness of different resistance genes and gene combinations in controlling Ugandan pathotypes of anthracnose and root rots. In addition to this, at least 20 bean breeding lines with dual resistance to anthracnose and root rot and broader resistance to anthracnose will be developed and taken up by the National Beans Research Program at NaCRRI for further testing and release as improved market-class varieties. Growers of these varieties will benefit through lower crop losses experienced on the farms which will translate into increased household bean production and thus incomes.

Acknowledgement

Our sincere thanks go to the Millennium Science Initiative (MSI), which, through the National Council of Science and Technology (UNCST) for funding this study. We also thank the Regional Universities Forum for Capacity Building (RUFORUM) for the administrative and technical support. We are grateful to Prof. Paul Gibson for the technical and moral support. Lastly we thank the Beans Research Program at the National Crops Resources Research Institute (NaCRRI) for the infrastructural and technical support and for providing the parental materials.

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