Research Application Summary

Assessing the effectiveness of pyramided genes in conferring broad resistance to bean anthracnose

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Abstract

Colletotrichum lindemuthianum is one of the most important constraints to common bean production in Uganda. The fungus is highly variable that single gene resistance alone is not adequate to offer effective and durable resistance among varieties. Earlier varieties known to be resistant have now succumbed to the disease. Therefore there is need for pyramiding of resistance genes as a strategy to circumvent the problem of pathogen variability. Hence, the aim of this study is to develop bean genotypes with broad resistance to bean anthracnose through a marker assisted gene pyramiding programme. The specific objectives of the study were to: a) identify diversity and physiological races of *C. lindemuthianum* in Uganda; b) to assess the effectiveness of pyramided genes in conferring resistance to bean anthracnose; c) to assess the effect of pyramided genes on plant agronomic traits; d) to determine mode of action and expression of pyramided resistance genes against *C. lindemuthianum*; and e) evaluate single anthracnose resistance genes dissected from parents G2333 and PI207262 using markers and introgressed in market class bean varieties K132 and NABE 4.

Key words: Broad resistance, *Colletotrichum lindemuthianum*, Gene pyramiding, marker assisted selection

Résumé

Colletotrichum lindemuthianum est l'une des contraintes les plus importantes pour la production du haricot commun en Ouganda. Le champignon est tellement variable que la résistance monogénique seule n'est pas suffisante pour offrir une résistance efficace et durable entre les variétés. Les variétés anciennes connues pour être résistantes ont succombé à la maladie. Par conséquent, il est nécessaire de pyramider les gènes de résistance comme une stratégie pour contourner le problème de la variabilité des agents pathogènes. Ainsi, le but de cette étude est de développer des génotypes de haricot avec

une grande résistance à l'anthracnose du haricot grâce à un programme de cumulation des gènes assisté des marqueurs. Les objectifs spécifiques de l'étude étaient les suivants: a) identifier la diversité et les races physiologiques de *C. lindemuthianum* en Ouganda; b) évaluer l'efficacité des gènes cumulés en conférant une résistance à l'anthracnose du haricot; c) évaluer les effets des gènes cumulés sur les caractères agronomiques des plantes, d) déterminer le mode d'action et d'expression des gènes de résistance cumulés contre *C. lindemuthianum*, et e) évaluer les gènes simples de résistance à l'anthracnose disséqués de parents G2333 et PI207262 à l'aide de marqueurs et incorporés dans les variétés de haricots de classe du marché K132 et NABE 4.

Mots clés: Grande résistance, *Colletotrichum lindemuthianum*, cumulation des gènes, sélection assistée d'un marqueur

Background

Colletotrichum lindemuthianum (Sacc. et. Magn) Lams. Scrib., possesses a high degree of genetic variability and it is thus implicated that single gene resistance alone is not adequate in offering effective and durable resistance against the pathogen. Until recent years most sources of resistance to bean anthracnose have sooner or later been overcome by the emergence of new pathotypes. There are a few available resistant varieties like G2333, Cornell 49-242, Tu and PI 207262 and moderately resistant local variety K131, but these have poor marketability, while the available market-class bean cultivars namely K132, NABE 4, NABE 13 and NABE 14 are all susceptible to anthracnose.

The ability of *C. lindemuthianum* to overcome incorporated resistances has greatly undermined previous breeding efforts leading to severe yield losses in bean varieties that had been previously released with a single gene resistance. This has created the need for pyramiding of resistance genes which have complementary spectra of resistance as a strategy to circumvent the problem of pathogen variability.

Therefore the current study aims at developing bean genotypes with broad resistance to bean anthracnose through a gene pyramiding programme aided by the use of SCAR markers for quick selection and gene tracking. The following objectives are being addressed; a) Identify diversity and physiological races of *C. lindemuthianum* in Uganda; b) assess the effectiveness of pyramided genes in conferring resistance to bean

anthracnose; c) assess the effect of pyramided genes on plant agronomic traits; d) determine mode of action and expression of pyramided resistance genes against *C. lindemuthianum*; and e) evaluate single anthracnose resistance genes dissected from parents G2333 and PI207262 using markers and introgressed in market class bean varieties K132 and NABE 4.

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 reported to cause up to 70% yield losses to farmers in Uganda when susceptible cultivars are used (Nkalubo, 2006). High pathogenic variability in *C. lindemuthianum* has been reported in the centre of origin of common bean. This has also been consistent in Uganda where different races have been confirmed (Leaky and Simbwa-Bunnya, 1971; Nkalubo, 2009). 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 anthracnose (Otsyula *et al*., 2005). Pyramiding these genes for resistance may be an effective strategy for controlling pathogen populations that pose a moderate risk of evolving virulent pathotypes. Gene pyramiding has been successfully applied in several crop breeding programmes, and many varieties and lines possessing multiple attributes have been produced. Depending on the trait and inheritance of targeted genes, however, gene pyramiding may require much labor, time and material resources. The development of modern plant molecular techniques and quantitative genetics in the last two decades has reduced this challenge and dramatically widened the applicability of gene pyramiding (Ye and Smith, 2008).

Study Description

Anthracnose diseased seed samples were collected from Kabarole, Kapchorwa, Kisoro, Maracha-Terego, Mbale, Oyam and Sironko districts and 51 isolates obtained on PDA and Mathur's media. Single spore isolates of *C. lindemuthianum* were placed on fresh Mathur's agar medium in a Petri dish and incubated at a controlled temperature between 22±2^{o}C for 4-7 days to allow the fungus enough time to produce conidial spores. For inoculation purposes, conidial spores were scrapped off the growth medium into a small amount of water to make a suspension. An inoculation concentration of 1.2 x 10^{6} conidia ml^{-1} containing 0.1% Tween 20 as a surfactant will be targeted.

To identify races, the binary system was used based on the sum of binary values assigned to each of the 12 differential cultivars proposed by Pastor-Corrales (1991).

Seven parental lines were used in the following gene pyramiding scheme; (((G2333 x PI207262) x RWR719) x K132, NABE4, NABE13 & NABE14). The parents G2333 and PI207262 were donors for anthracnose resistance genes, parent RWR719 was a donor for *Pythium* root rot resistance gene, while the parents K132, NABE4, NABE13 and NABE 14 were susceptible market class varieties to be improved. DNA extraction and PCR amplification (Fig. 1) and marker assisted selection (MAS) were carried out during the pedigree and fixation stages up to F_4 (Fig. 2). Sample DNA was amplified using standard PCR protocol with minor modifications. PCR products were separated on 1.2% w/v Agarose gel and visualized under Ultra Violet (UV) light following staining with ethidium bromide. To evaluate the effectiveness of the single genes and pyramided genes against different anthracnose races, F_4 pyramided families will be screened with a range of anthracnose races in a controlled screen house; using detached leaf method. Severity will be scored using the 1-9 scale and the data will be subjected to ANOVA using GenStat Discovery 14th Edition. A qRT-PCR will be used to study expression of pyramided genes. A screen house agronomic study will be carried out to determine effect of pyramided genes on plant agronomic traits.

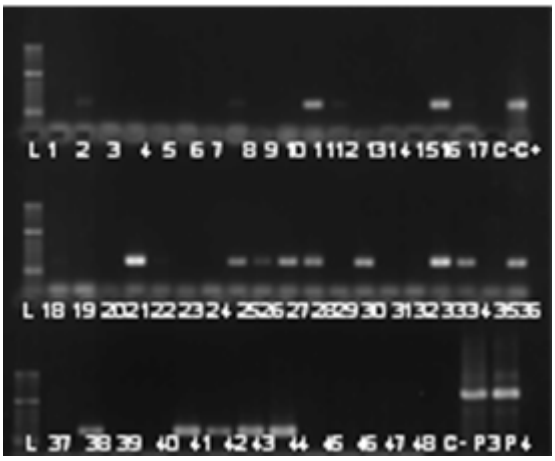

Figure 1. (d) DNA amplification of SAB3 primer linked to the Co-5 gene. Lanes: *L* 2000bp ladder (Hyperladder II, 500 lanes); *1-36* lines from the cross ((G2333xPI207262)xRWR719); *C-* negative control; *C+* positive control.

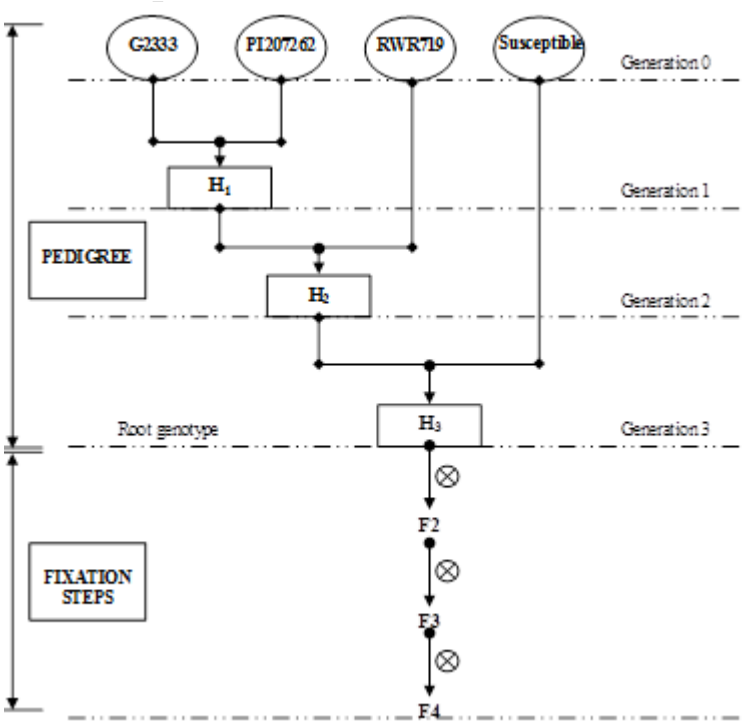

Figure 2. Gene pyramiding scheme used.

Research Application

Twenty seven races were identified using this method (Table 1). Information about the effectiveness of different resistance genes and gene combinations in controlling Ugandan anthracnose races will be generated and used by bean breeders to develop varieties with broader resistance. Over 100 bean breeding lines with dual resistance to anthracnose and *Pythium* root rot and broad resistance to anthracnose have been generated and will be availed to the National Beans Research Programme at National Crops Resources Research Institute (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.

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Table 1. Race characterization using binary system.

Race*	Differential cultivars and their respective resistance genes [a]												Isolates
	1	2	3	4	5	6	7	8	9	10	11	12	
0	-	-	-	-	-	-	-	-	-	-	-	-	38A, 59A, 57A, 25A
1	+	-	-	-	-	-	-	-	-	-	-	-	82A
42	-	+	-	+	-	+	-	-	-	-	-	-	85A
81	+	-	-	-	+	-	+	-	-	-	-	-	92A
128	-	-	-	-	-	-	-	+	-	-	-	-	36A
352	-	-	-	-	-	+	+	-	+	-	-	-	84A
386	-	+	-	-	-	-	-	+	+	-	-	-	61A
503	+	+	+	-	+	+	+	+	+	-	-	-	88A
704	-	-	-	-	-	-	+	+	-	+	-	-	34A
713	+	-	-	+	-	-	+	+	-	+	-	-	86A
767	+	+	+	+	+	+	+	+	-	+	-	-	69A
784	-	-	-	-	+	-	-	-	+	+	-	-	56A
1023	+	+	+	+	+	+	+	+	+	+	-	-	62A
1094	-	+	+	-	-	-	+	-	-	-	+	-	12A
1169	+	-	-	-	+	-	-	+	-	-	+	-	72A
1175	+	+	+	-	+	-	-	+	-	-	+	-	91A
1334	-	+	+	-	+	+	-	-	+	-	+	-	94A
1471	+	+	+	+	+	+	-	+	+	-	+	-	90A, 100A
1527	+	+	+	-	+	+	+	+	+	-	+	-	16A, 99A
1791	+	+	+	+	+	+	+	+	-	+	+	-	41A
1834	-	+	-	+	-	+	-	-	+	+	+	-	81A
2023	+	+	+	-	-	+	+	+	+	+	+	-	007A
2039	+	+	+	-	+	+	+	+	+	+	+	-	97A
2045	+	-	+	+	+	+	+	+	+	+	+	-	95A, 40A
2047	+	+	+	+	+	+	+	+	+	+	+	-	65A, 08A, 64A, 75A, 98A, 52A, 55A, 37A, 46A,71A
2479	+	+	+	+	-	+	-	+	+	-	-	+	83A
4095	+	+	+	+	+	+	+	+	+	+	+	+	66A, 63A, 44A, 67A, 76A, 28A, 001A, 77A, 73A, 96A

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