Research Application Summary

Marker assisted introgression of *Phytophthora capsici* resistance genes into locally adapted pepper variety cv. scotch bonnet

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Abstract

Phytophthora capsici, a soil born fungus, is one of the most important constraint to hot pepper production in Uganda, causing root rot and wilt disease. Current management approaches that are mainly cultural and chemical do not provide adequate protection against the disease. Consequently, a lot of the crop is lost. This study is broadly aiming at developing resistance against the disease. Specific objectives of the study are: (i) to test the effectiveness of molecular markers in aiding the introgression of the *Phyto* 5.2 QTL into susceptible varieties, and (ii) to determine the contribution of the *Phyto* 5.2 QTL towards resistance to *P. capsici* in pepper. A resistant line was crossed with a susceptible variety. F_1s were screened using a SCAR and SSR markers reported to be linked to *Phyto* 5.2, a major QTL for resistance to the disease. A total of seven SSR markers were tested in addition to the SCAR. The SCAR marker was unfortunately not polymorphic, while only one SSR marker is promising. F_2s and backcross 1 populations will be phenotypically evaluated to determine any relationship between observed resistance and presence of SSR marker.

Key words: Hot pepper, Phyto 5.2 SCAR markers, *Phytophthora capsici*, SSR marker

Résumé

Phytophthora capsici, un champignon né dans le sol, est l'une de plus importantes contraintes pour la production du poivre pimenté en Ouganda, provoquant la pourriture des racines et la maladie du flétrissement. Les approches de gestion actuelles qui sont essentiellement culturelles et chimiques ne fournissent pas une protection adéquate contre la maladie. Par conséquent, une grande partie de la récolte est perdue. Cette étude vise largement à développer une résistance contre la maladie. Les objectifs spécifiques de l'étude sont les suivants: (i) tester l'efficacité des marqueurs moléculaires en aidant l'incorporation des gènes de *Phyto*5.2 dans les variétés sensibles, et (ii) déterminer la contribution de *Phyto*5.2 QTL pour la résistance à *P. capsici* dans le poivre. Une lignée de résistance a été

croisée avec une variété sensible. F1s ont été examinés à l'aide des marqueurs SCAR et SSR signalés être liés à *Phyto*5.2, un QTL important pour la résistance à la maladie. Un total de sept marqueurs SSR ont été testés en plus du marqueur SCAR. Ce dernier n'a malheureusement pas été polymorphe, alors que seulement un marqueur SSR est prometteur. F2s et les populations 1 croisées après seront phénotypiquement évaluées afin de déterminer une relation entre la résistance observée et la présence des marqueurs SSR.

Mots clés: Poivre pimenté, marqueurs SCAR *Phyto* 5.2, *Phytophthora capsici*, marqueur SSR

Background

Phytophthora capsici, a soil born fungus, which causes fruit root and crown rot, has been identified as one of the most important constraint to hot pepper production in Ugandan commercial production fields (Tusiime *et al*., 2010). Cultural, chemical and biological approaches (Minamiyama *et al*., 2007) have been used to manage the *Phytophthora* root rot and wilt disease. These control measures however, do not provide adequate protection against full-scale epidemics (Hausbeck and Lemour, 2004). Therefore host resistance would be the most appropriate method for control of *Phytophthora* root rot and *blight* disease.

Resistance in *Capsicum* to *Phytophthora capsici* is genetically and physiologically complex with reports of single, two and multiple gene system being involved (Quirin *et al.,* 2005). Thus, the use of molecular markers may be important in enhancing selection efficiency to allow for rapid screening of individuals with resistance genes during breeding.

Quirin *et al*. (2005) developed a SCAR marker that amplifies bands only in highly resistant pepper plants. They used this SCAR to trace the major QTL (*Phyto* 5.2 QTL) for resistance against *Phytophthora* root rot and wilt in segregating progeny. Minamiyama *et al*. (2007) also mapped this SCAR marker as nearly the same locus as SSR markers denoted CAMS 051 and CAMS 163. This set of markers can potentially identify highly resistant plants, and can expedite the process of resistance breeding while maintaining the desirable traits in pepper. There is however need to validate their utility before they can be deployed for full scale breeding for resistance against *P. capsici* in pepper.

Literature Summary

Several sources of resistance to *P. capsici* have been identified in *C. annuum* lines such as Criollo de Morelos 334 (CM334), NY07-8001, NY07- 8006, and NY07- 8007 (Foster and Hausbeck, 2010). Utilisation of these as a source of resistance may however be limited because of the poor crossability between *Capsicum chinense* and *Capsicum annuum*.

Selection for many chromosomal regions at once without altering some of the highly desirable attributes of pepper has been a challenge in the breeding programmes (Ogundiwin *et al*., 2005; Quirin *et al*., 2005). Marker Assisted Selection (MAS) for one or a few most critical QTL in combination with phenotypic analyses has been proposed as an easier way to introgress high levels of resistance to *P. capsici* in pepper (Thabuis *et al*., 2004).

Elsewhere, molecular markers have been developed such as Simple sequence repeats (SSRs), Random amplified polymorphic DNAs (RAPDs), Amplified fragment length polymorphism (AFLPs), and Sequence characterized amplified regions (SCARs) (Ogundiwin *et al*., 2005: Quirin *et al*., 2005) to aid in the selection of resistant hot pepper plants.

Similar resistance breeding studies using MAS have successfully been done in other crops such as maize, soybean, tomato, pepper among others (Semagn *et al*., 2006). Thus, the use of molecular markers will enhance selection efficiency and allow for rapid screening of hot pepper plants with resistance gene, thus faster variety development and release. Therefore there is need to validate the applicability of these markers for selection breeding under the Ugandan situation.

Study Description

Screen house studies were conducted at Makerere University Agriculture Research Institute Kabanyolo (MUARIK) for two consecutive seasons during 2011. In the first season, a resistant hot pepper variety (PI accession) imported from USA, together with the susceptible scotch bonnet varieties were planted and crossed at flowering to raise F_1 seedlings. In season two, the F_1 seedlings were planted and up to 115 accessions screened for resistance using SCAR markers. DNA was extracted from them using CTAB method (Doyle and Doyle, 1990). It was then amplified using Phyto 5.2 SCAR (Quirin *et al*., 2005) and seven of the SSR markers developed by Minamiyama *et al*. (2007). PCR products were separated by electrophoresis on 3% agarose gel stained with Gel Red at 110V for 2 hrs and gel

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images taken using a BioDoc System. For each marker, associated DNA bands were scored for presence or absence.

The SCAR marker developed by Quirin *et al*. (2005) was not polymorphic in this study.When used to screen the F1s, the banding pattern was the same in the resistant and susceptible parents. In both cases the expected DNA fragment (740 bp) was amplified (Fig. 1). Of the seven SSR markers tested, only CAMS 051 was polymorphic (Fig. 2).

In this study, the SCAR marker was not polymorphic and therefore cannot be useful in breeding for resistance to the disease. This could be a result of the differences in populations

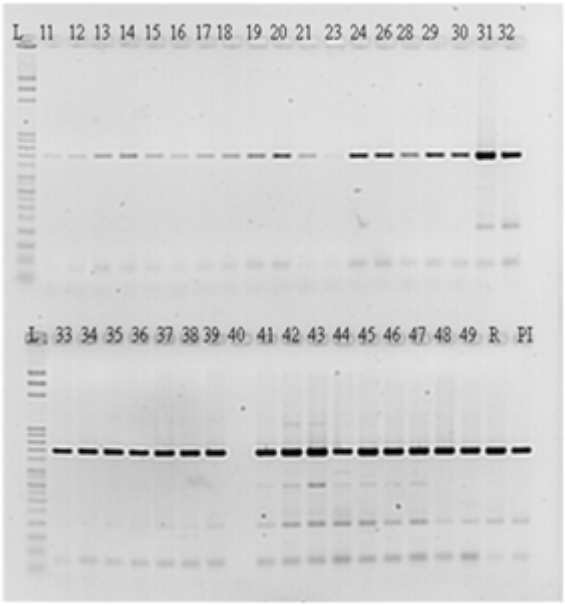

Figure 1: Detection of the 5.2 Phyto SCAR in F1 pepper progeny derived from a cross of resistant and susceptible pepper. L, Ladder; PI, (Resistant parent); R, Susceptible parent; 11 to 49, F1 accessions.

Figure 2: Detection of the 5.2 Phyto QTL using a linked CAMS 051 SSR marker in F1 pepper progeny derived from a cross of resistant and susceptible pepper. S = susceptible parent: R = Resistant parent; 1-4 = F1 accessions.

in which it was originally mapped and our study population. The CAMS 051 SSR marker is promising. Its performance should be confirmed with phenotypic studies.

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