SCAR MARKER LINKED TO THE CO-2 GENE FOR THE ANTHRACNOSE

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Keywords: *Phaseolus vulgaris L., Anthracnose, SCAR*

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important food legume consumed worldwide. Beans provide an important source of protein, vitamins and minerals for human diets, especially in developing countries (Broughton et al., 2003). Anthracnose, caused by *Colletotrichum lindemuthianum*, is a serious seed borne, hemibiotrophic fungus capable of inducing complete yield loss in susceptible bean genotypes. Due to the high degree of pathogenic variability of the fungus and the continual emergence of new races, genetic resistance in the host is not durable (Kelly et. al., 2004).

Young and Kelly (1996) have identified two RAPD markers, $OQ4_{1440bp}$ and $B355_{1000bp}$ flanking the Co-2 gene and these markers mapped at 2.0 to 5.4 cM in Andean and from 5.5 to 7.7 cM in Middle American backgrounds. Major limitation in RAPD is repeatability, because PCR reactions are very sensitive to factors such as annealing temperature, template DNA concentration *etc*. Under such circumstances, cloning and further sequence characterization of the RAPD fragments and designing longer and specific oligonucleotide primers (Sequence Characterized Amplified Regions – SCARs) to verify its linkage to the targeted trait in a segregating progeny would be more reliable and will facilitate its application in MAS since, SCAR are codominant in nature and are highly reproducible and stable (Tanksley et. al., 1989). Hence, the present study was conducted with the objective of developing a SCAR marker for the Co-2 gene is based on widespread deployment of these markers in bean breeding programmes worldwide.

Methodology

*DNA extraction:*DNA was extracted from few advanced lines of dry bean using a mini-prep method with slight modifications. *RAPD Analysis:*Total volume of PCR mixture was 26 µl, which contained 5 units of Taq. polymerase enzyme (invitro gene brand), 4 µl dNTP (5mM mix), 2.5 µl 10X PCR buffer, 2.5 µl (50mM) $MgCl_2$, 3 µl primer (10 ng/ µl of OQ4; Operon Technologies Inc., Alameda, USA) and 3 µl (10 ng/ µl) of genomic DNA template.

*Cloning and sequencing of the RAPD fragment:*The putative marker amplified by the random primer OQ4 was excised from 1.4% agarose gel with sterile gel slicer (Fig.1) and purified using the QIA quick gel extraction kit (Qiagen, Maryland, USA). DNA insert was sequenced on an ABI 310 automated sequencer using vector specific universal promoter, M13 reverse and forward, and T7 primers.

*SCAR primer designing and validation:*Based on the sequence of unique RAPD amplicon a pair of SCAR oligonucleotide primers which could amplify 1440 bp of the genomic DNA was designed. The SCAR primer pair was used for PCR amplifications of genomic DNA. The SCAR primers were validated using all varieties known to carry the Co-2 gene. Sequence data of SQ4 was submitted to BLASTX search of *Phaseolus vulgaris* database.

Discussion and Conclusion

*DNA extraction and RAPD:*The DNA extraction procedure yielded 500-700ng of DNA. An absorbance (A_{260}/A_{280}) ratio of 1.6-1.8 indicated insignificant levels of contaminating proteins and polysaccharides. OQ4 consistently amplified an intense 1440 bp band that was unique to Cornell 49-242, Balckhawk, Chinook 2000, K03601 and this band was not observed in Montcalm (Fig. 1).

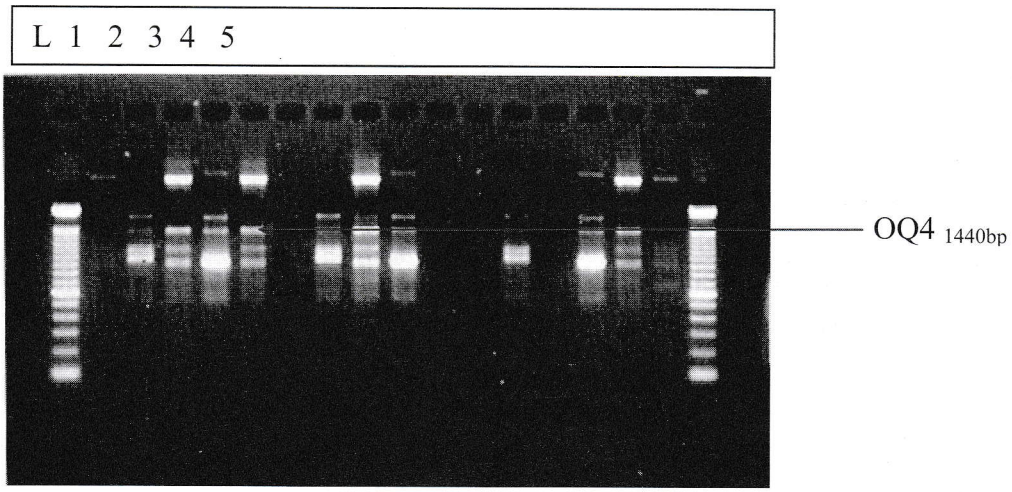

Figure 1:Banding pattern of dry bean lines for OQ4 Primer (L- Ladder DNA, Lane 1- Montcalm, Lane 2- Cornell 49-242, Lane 3– Balckhawk, Lane 4 – Chinook 2000 and Lane 5- K03601).

Cloning and Sequencing of polymorphic Band:*Phaseolus vulgaris* specific 1440 bp polymorphic band was cloned into pCR® 2.1- Topo® (invitrogen, USA) vector. The selected white colonies contained the required recombinant construct as was confirmed by reamplification with OQ4.

*Validation of SCAR primers:*Thedesigned SCAR primer pair was used to amplify the genomic DNA from Cornell 49-242, Balckhawk, Chinook 2000, K03601 and Montcalm. A single, distinct and brightly resolved band of 1440bp was obtained only with the Cornell 49-242, Balckhawk, Chinook 2000, K03601 while no amplification product was obtained with Montcalm (Fig 2). The best temperature for annealing of both primer was 59°C.

The SCAR primers designed using the sequence variation was found to be specific for known lines of dry bean which carry Co-2 gene, making the technique more stringent and specific when compared with RAPD marker. Identifying specific molecular markers linked to the anthracnose resistance will facilitate indirect selection of such complex traits through marker –assisted selection (MAS).

The results have thus identified a SCAR marker $SQ4_{1440bp}$ co-segregating with anthracnose resistance gene Co-2. Further studies on mapping of the SCAR marker on chromosome linkage map by genotyping several lines of dry bean population segregating for anthracnose resistance will validate the significance of the marker for its use in MAS for anthracnose resistance improvement in dry bean (*Phaseolus vulgaris* L.).

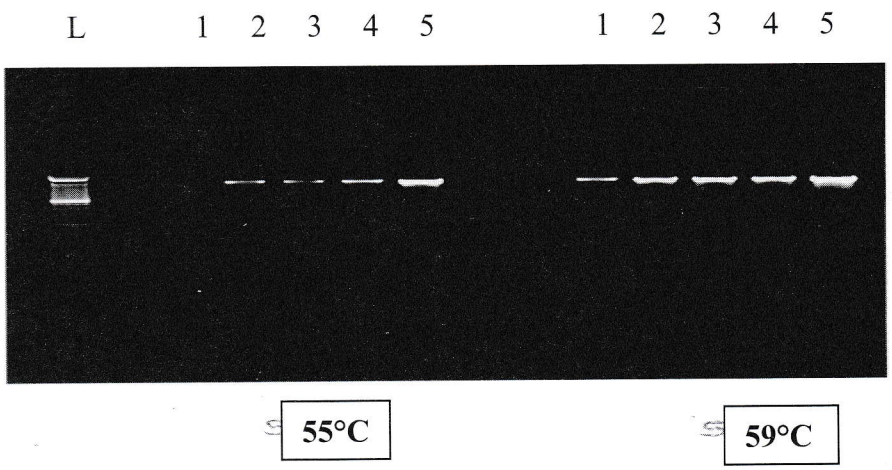

Figure 2: SCAR amplification of dry bean lines for SQ4 at 1440 bp at annealing temperature 55°Cand 59°C. (L- Ladder DNA, Lane 1- Montcalm, Lane 2- Cornell 49-242, Lane 3– Balckhawk, Lane 4 – Chinook 2000 and Lane 5- K03601).

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