ABSTRACT

Title of the Thesis	:	Identification of causal SNPs in promoter sequence of *Co-2* and development of functional markers for anthracnose resistance in common bean (*Phaseolus vulgaris* L.)
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Abstract

Anthracnose caused by the fungus *Colletotrichum lindemuthianum* is one of the most devastating diseases of common beans causing yield loss up to 100 per cent. The study was conducted to identify single nucleotide polymorphism sequences (SNPs) in the promoter region of the *Co-2* gene, to identify causal SNPs linked to anthracnose disease resistance using phenotypic and SNP data and to develop functional marker to differentiate the alleles of causal SNPs and validation for anthracnose resistance. In the present study, the *Colletotrichum* isolates collected from Doda and Rajouri districts of Jammu and Kashmir were identified based on micromorphology and cultural characteristics. Multigene sequence data (ITS, GAPDH, CHS-1, HIS3, ACT and TUB2) and phylogenetic analyses (ML) of the isolates authenticated them as *Colletotrichum lindemuthianum.* Results showed that all the tested isolates of *C. lindemuthianum* were pathogenic to *Phaseolus vulgaris cv.* Bhaderwah-*Rajmash* (local name). Besides this, 87 collected diverse genotypes of common bean were screened morphologically against this isolate under epiphytotic conditions. Most of the genotypes showed high resistance and moderate resistance. Identified resistant genotypes were further confirmed by screening the common bean genotypes for *Co-2* gene using SCAR marker $SCAreoli_{1300/1000}$. Out of these genotypes, 10 common bean genotypes viz EC-500250, IC-274530, S1, S3, EC-398527, EC-398565, BR36, EC-398591, KB12, EC-121013 genotypes indicated the presence of resistance allele. Using NCBI data, promoter region of *Co-2* gene was identified and marker was developed using Primer 3 software. Three anthracnose resistant and susceptible genotypes were amplified, cloned and sequenced for identification of SNPs associated to disease resistance. Twenty-three SNPs were identified at the regulatory motifs regions using Nsite (www.softberry.com) at positions 30, 32, 38, 39, 188, 223, 228, 326, 329, 475, 477, 481, 499, 503, 1017, 1019, 1024, 1369, 1373, 1469, 1471, 1472 and 1476. Fourteen SNPs were found to be strongly associated for disease resistance using phenotypic and genotypic data. Allele specific marker with forward sequence 5' TGGATGATGTTTGGA ACGAA 3' and reverse sequence 5' CCTTCCCACTCTCCAACAGA 3' was developed using dCAPS Finder 2.0 software from SNP at position 477. The identified allelic marker can be used for transferring anthracnose disease resistance allele from highly resistant genotypes into susceptible cultivated varieties of common bean using marker-assisted selection (MAS) methods.