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CLONING, MAPPING, AND BREEDING FOR DISEASE RESISTANCE IN COMMON BEANS (*Phaseolus vulgaris* L.)

by

Nedim Mutlu

A DISSERTATION

Presented to the Faculty of

The Graduate Collage at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Interdepartmental Area of
Major: Horticulture and Forestry

Under the Supervision of Professor Dermot P. Coyne

Lincoln, Nebraska

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DISSERTATION TITLE

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(Phaseolus vulgaris L.)

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Nedim Mutlu

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GRADUATE COLLEGE
UNIVERSITY OF NEBRASKA

CLONING, MAPPING, AND BREEDING FOR DISEASE RESISTANCE IN COMMON BEAN (*Phaseolus vulgaris* L)

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University of Nebraska, 2002

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Resistance genes containing nucleotide binding site (NBS)-leucine rich repeats (LRR) are the most prevalent types of resistance (R) genes in plants. Kinase-1a domain, in NBS region, is conserved in a few superfamilies, including all NBS-LRR type plant resistance genes. The objectives of this study were: to clone and map members of the kinase-1a gene family, to develop PCR based R-gene specific markers for common bean (*Phaseolus vulgaris* L), and transfer the quantitative trait loci (QTL) for resistance to common bacterial blight (CBB), caused by *Xanthomonas campestris pv. phaseoli*, from exotic germplasm XAN-159 to two commercial varieties, pinto 'Chase' and great northern (GN) 'Weihing'.

Degenerate primers were used from the most common peptide sequences of kinase-1a and hydrophobic domains (HD) of known NBS-LRR type R genes and EST database. Kinase-1a genes amplified from a cDNA bulk, cloned and mapped via restriction length polymorphism (RFLP). Twenty degenerate primers were used to screen parents BAT 93 and Jalo EEP558 for RGA mapping. Twenty-six kinase-1a gene through RFLP and 32 RGA via PCR were mapped in the common bean recombinant inbred (RI) population BAT93 x Jalo EEP558 where 68 and 72 lines were used,

respectively. No kinase-1a gene mapped on linkage group B8 or B10, and no RGA mapped on B4. The linkages to known resistance genes or QTL and important agronomic traits were shown with both kinase-1a and RGA markers. The total of 58 new markers are expected to aid marker assisted selection and future gene cloning studies in common bean.

CBB resistance was confirmed, both with greenhouse screening and under natural infection in the field, in advanced BC5 lines for pinto'Chase' and BC3F2 plants for GN.'Weihing'. The linkage to *V* locus that is associated with small seed size and black seed coat color was broken in some BC4 and BC5 pinto 'Chase' lines where high resistance to CBB was combined with acceptable seed size and pinto seed coat color.

THIS DISSERTATION IS DEDICATED TO MY ADVISER PROFESSOR **Dr. Dermot P. COYNE**, WHO PASSED AWAY April 12, 2002, ELEVEN DAYS BEFORE THE DEFENSE DATE OF THIS DISSERTATION.

I HAVE HAD THE HONOR AND THE PRIVILAGE OF KNOWING HIM, STUDYING UNDER HIS GUIDIENCE, AND I WILL ALWAYS LOOK UP TO THE HIGH VALUES HE FOLLOWED AS LONG AS I LIVE.

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CHAPTER I

MAPPING FOR DISEASE RESISTANCE IN COMMON BEAN (*Phaseolus vulgaris* L)

Additional Index Words. NBS-LRR, RGA, Common bean, mapping.

Abstract

Resistance genes containing nucleotide binding site (NBS)-leucine rich repeats (LRR) are the most prevalent types of resistance (R) genes in plants. Kinase-1a domain, in the NBS region, is conserved in a few superfamilies, including all NBS-LRR type plant resistance genes. The objectives of this study in section I were: to clone members of the kinase-1a gene family from a bulk of cDNA derived from challenged (with *Xanthomonas campestris pv phaseoli*) plants and to determine genome-wide distribution of this gene family relative to known traits and/or genes on the integrated common bean map, and in section II: to develop R-gene specific, PCR based markers for common bean (*Phaseolus vulgaris* L). The second part is a resistance gene analogs (RGA) mapping study.

Degenerate primers were used from the most common peptide sequences of kinase-1a and hydrophobic domains (HD). Known NBS-LRR type R genes and EST database were used to determine the most common peptide sequences conserved among R genes for the two domains. For kinase-1a gene family mapping, genes were amplified from a bulk cDNA sample, cloned and mapped via restriction length polymorphisms (RFLP). For RGA mapping, 20 degenerate primers were used to screen bean lines, BAT 93 and Jalo EEP558. Twenty-six kinase-1a genes and 32 RGA were mapped by RFLP and via PCR in the common bean recombinant inbred lines (RIL) population BAT93 x

Jalo EEP558 where 68 and 72 lines were used, respectively. No kinase-1a genes were mapped on linkage group B8 or B10, and no RGA was mapped on B4. The linkages to known resistance genes or QTL and important agronomic traits were shown with both kinase-1a and RGA markers. The total of 58 new markers are expected to aid marker assisted selection and future gene cloning studies in common bean.

SECTION I

Many kinase-1a containing genes co-localize with resistance and domestication trait genes/QTLs in common bean (*Phaseolus vulgaris* L.)

Abstract

The kinase-1a domain is present in a few superfamilies, including all NBS-LRR type plant resistance genes. In this study, members of the kinase-1a gene family were cloned and sequence structures were characterized. Localization of these genes was determined relative to known agronomic traits on the integrated common bean map. The amino acid sequence GVGKTT of the kinase-1a domain is highly conserved in NBS-LRR type dicot resistant genes. Degenerate primers corresponding to the kinase-1a GVGKTT sequence for the 5'end and nine different T-primers or a hydrophobic domain (HD) GLPLAL sequence for the 3' end were used to amplify target sequences from a bulk of cDNA derived from bacterial pathogen challenged plants. An average of 108 bands were observed for each primer combination in kinase-1a and T-primers and a total 117 bands were found for kinase-1a and HD primers. Additional T-primers amplified 42 additional unique bands. A total 150 and 117 bands were cut out of the gel, amplified and cloned in a T-A cloning system for kinase-1a and T-primers and kinase-1a and HD primers, respectively. Of the 21 kinase-1a and HD clones, 10 were unique and 11 were common genes with kinase-1a and T-primer groups. Sequence analysis showed 104 unique cDNAs of which 61 were found structurally to be R-genes of plants. A total of 26 kinase-1a genes were distributed on all linkage groups except B8 and B10. These genes mapped

close to domestication traits, pathogenesis related genes, resistance genes and QTLs for resistance. These kinase-1a markers are expected to be useful in future cloning efforts of proximal QTLs/genes.

Introduction

A nucleotide binding site (NBS) containing protein is believed to activate a kinase, or act as a G-protein, each being part of a signal transduction pathway (Bent, 1996). Plants have developed systems to quickly and accurately transmit stimuli from the external environment and to adjust their metabolic pathways by modulating the expression of sets of genes. Activation and/or inactivation of appropriate genes in response to particular stimuli are mediated through well-tuned signal transduction systems in which protein phosphorylation cascades play crucial roles (Crews and Erikson, 1993; Stone and Walker, 1995). Domains are homologous portions of protein sequences that are encoded in different genes and have survived the evolutionary tests of time without fragmentation. The presence of these domains in diverse gene families demonstrates that evolution has constantly fused and divided genes, using a repertoire of pre-existing components. It has been suggested that structurally conserved modules that are found in distantly related proteins are descended from a limited number of ancestral sequence motifs (Doolittle, 1995). Although the protein repertoires of distantly related species differ greatly, their domain repertoires do not. Whether exon shuffling and/or other mechanisms of genetic recombination can explain their widespread distribution is still debated (Shutov et al., 1999). Proteins are constructed from a limited number of motifs that include regulatory or catalytic sites.

The phosphate binding loop (P-loop) is one motif commonly found in ATP/GTP binding proteins (Walker, 1982) and kinase-1a is one of the structural P-loop motifs found in a few specific superfamilies (Kinoshita et al., 1999). Nucleotide binding proteins contain several motifs that together form the active site, the kinase-1a comprising only one of these motifs. Additional nucleotide binding motifs, such as kinase-2 and kinase-3 motifs, generally reside C-terminal to the kinase-1a (Traut, 1994). Kinase-1a is one of the nucleotide binding site motifs that function in binding of phosphates of the ligand (Traut, 1994). The kinase-1a motif is a glycine-rich sequence that characteristically folds into a flexible loop between an α-helix and a β-strand, with the invariant lysine at the first position of the α-helix and interacts with one of the phosphate groups of the bound nucleotide.

Conservation of the amino acid sequence at the kinase-1a domain surprisingly relates to defense mechanisms in both plant and mammalian systems. In mammals for example, kinase-1a containing genes Apaf 1 and Ced-4 regulate initiation of apoptotic cell death (Li and Chory, 1997). The mammalian NOD1 and NOD2 genes which share homology with the NBS-LRR superfamily of plant disease resistance proteins function as intracellular receptors for bacterial Lipopolysaccharides (LPS) and play a key role in innate immunity (Inohara et al., 1999). Interestingly, NOD2 seems to be associated with Crohn's disease in humans (Ogura et al., 2001). Also NOD family proteins are structurally homologous to the apoptosis regulators APAF1/CED4 and to NBS-LRR plant disease resistance proteins. The NOD family contains an NH_2 –terminal specific enzyme type caspase recrutiment domain (CARD), a central NBS domain, and C-terminal LRR domains. The human genome sequence suggests about 30 NOD gene

homologs, expressing NBS-LRR domains but with potentially different NH_2 –terminal domains (Staskawicz et al., 2001).

The kinase-1a domain (NBS) is common in most of the cloned R genes. The conserved nature of the kinase-1a domain in many plant NBS-LRR type resistance genes was utilized for PCR amplification of fragments, cloning, sequencing and mapping studies in order to isolate and characterize homologs in potato (Leister et al., 1996), soybean (Kanazin et al., 1996; Yu et al., 1996), common bean (Rivkin et al., 1999), wheat and rice (Chen et al., 1998) and lettuce (Shen et al., 1998).

Common bean (*Phaseolus vulgaris* L.;2n=2x=22) is one of the legume species with the smallest genomes, with 657 Mbp or 0.66 pg/haploid genome (Aramuganathan K, 1991). Reassociation kinetics suggests that 60% of the genome is comprised of single copy sequences (Talbot et al. 1984). The first RFLP map of common bean was constructed with 250 markers distributed among 11 linkage groups (Vallejos et al., 1992). Later smaller maps were reported (Adam-Blondon et al., 1994; Nodari et al., 1993b), with 152 markers in 15 linkage groups and 157 markers (51 RFLPs and 100 RAPD, and 6 other markers) in 12 linkage groups, respectively. Simple sequence repeat (SSR) markers were also integrated into the bean map (Yu et al., 2000).

Mutations in qualitative R genes (hypersensitive type response to different groups of pathogens) such as rice Xa21, flax M, and tomato I2, altered the HR and resulted in a partial resistance phenotype (Anderson et al., 1997; Ori et al., 1997; Wang et al., 1998). These mutated-R gene phenotypes were comparable to quantitative resistance controlled by multiple genes. A QTL for partial resistance to cucumber mosaic virus (CMV) with an additive effect was found linked or allelic to one NBS-type family in pepper

(*Capsicum annum*) (Pflieger et al., 1999) and QTLs with epistatic effects were also reported at several NBS loci in the same study. QTLs for resistance and R genes reside as tightly linked clusters on chromosomes V, XI, and XII of potato (Gebhardt and Valkonen, 2001) and soybean linkage group (LG) F (Jeong et al., 2001).

Genomic clustering of resistance genes was observed when NBS-LRR sequences were mapped (Yu et al., 1996); (Leister et al., 1996); (Collins et al., 1998). Genes for resistance to different pathogens formed clusters at three genomic regions in potato (Gebhardt and Valkonen, 2001). Dm genes of lettuce (Meyers et al., 1998), the Cf genes of tomato (Dixon et al., 1998; Jones et al., 1994), and Mla genes of barley (Jorgensen, 1992) reside within the NBS-LRR gene family clusters.

Resistance genes and resistance gene analogs (RGA) were also reported to co-localize in many crops (Aarts et al., 1998; Gentzbittel et al., 1998; Kanazin et al., 1996; Leister et al., 1996; Shen et al., 1998; Speulman et al., 1998; Yu et al., 1996). QTLs for resistance and RGAs were also shown to co-localize in pepper (Pflieger et al., 1999), in soybean (Kanazin et al., 1996), and in sunflower (*Helianthus annus*) (Gentzbittel et al., 1998). Hovewer, none of the RGA loci co-segregated with disease resistance loci in cowpea (*Vigna unguiculata* L.), a legume closely related to common bean (Ouedraogo, 2002).

Kinase-1a containing genes were cloned from a cDNA bulk derived from plants challenged (with *Xanthomonas campestris pv. phaseoli*). Thus, these genes are expected to be functional, unlike RGAs cloned from genomic DNAs. And low copy number genes were also cloned due to high-resolution separation of the target product. In this paper, we