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Full Length Research Paper

Peptide sequences from seed storage proteins of tepary bean (*Phaseolus* acutifolius) accession G40199 demonstrate the presence of multiple variants of APA proteins

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Proteomic analysis of the expression of seed storage proteins was performed in wild tepary beans (P. acutifolius) accession G40199. The interspecific backcross progenies were obtained from the cross between G40199 and cultivated common bean (*Phaseolus vulgaris*) cultivars ICA Pijao and/or Rojo. Mature seeds from interspecific backcross progenies and G40199 were used to isolate seed storage proteins. Seed storage profiles obtained by SDS-PAGE, and bands were isolated and used for MS/MS-ES peptide sequencing and analysis. Peptide peaks and their amino acids sequences demonstrated that both the interspecific backcross progenies and G40199 contain arcelins and arcelin-like (ARL2) proteins that are co-expressed with other proteins of the arcelin, □-amylase inhibitor and phytohemagglutinin (APA) locus. Arcelin and ARL2 proteins previously obtained from a wild tepary bean accession of this APA were the predominant types detected in the wild tepary bean accession G40199 demonstrating the presence of multiple arcelin-like proteins as the major seed storage proteins. The occurrence of multiple variants of the APA seed storage proteins in a single accession presents an important opportunity for exploration of antibiosis proteins in breeding for resistance to bean bruchids, major storage pests in common beans in the tropics.

Key words: APA locus, arcelin, Acanthoscellides obtectus, Zabrotes subfasciatus, peptides, tepary beans, bruchid resistance.

INTRODUCTION

Various species of the genus Phaseolus contain a family of related lectins and lectin-like proteins (LLP) that are associated with antibiosis activity against seed storage pests. These proteins accumulate in the seed tissues and are the product of an orthologous multigene family that has evolved through extensive duplication events, and has been coopted for the purpose of plant defense against bruchids (Sparvoli et al., 2001; Lioi et al., 2003). Phytohaemagglutinin (PHA) is the major lectin of beans. PHA functions as a carbohydrate binding protein that defends plant against predation by most organisms, though less effectively against cowpea weevil-Collosobruchus maculatus (Murdock et al., 1990).

The three proteins - PHA-arcelin-α-AI constitutes a complex locus for the LLPs that have arisen through duplication of a single gene of an ancestral lectin (Nodari et al., 1993; Van Damme et al., 1998; Sparvoli et al.,1998; 2001). These LLPs are also found in certain

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accessions of lima and tepary bean and have been described as arcelin-like (AL) and alpha amylase inhibitor-like (α-AIL) proteins (Mirkov et al., 1994; Sparvoli et al., 1998; 2001; Sales et al., 2000; Lioi et al., 1999; 2003). The differential expression of lectins and lectin-like seed proteins among legumes demonstrates a divergence in function related to the evolution of legume species (Lioi et al., 2006).

As part of a search for high levels of resistance to bruchids, Cardona et al. (2005) identified the accession G40199 of tepary bean (Phaseolus acutifolius), which confers strong resistance to the bruchids; *Acanthoscellides obtectus* and *Zabrotes subfasciatus*. Resistance mechanism(s) residing in this accession have been described to be associated with the presence of a major 33 kDa storage protein corresponding to molecular size of lectins and was successfully transferred into selected genotypes of common beans by interspecific hybridization (Kusolwa, 2007; Mbogo, 2009). The protein was hypothesized to be either one or both of the three LLP seed defense proteins of the lectin family - arcelins (ARL), phytohaemagglutinins (PHA) or alpha amylase-inhibitors (□-AI).

Characterization of genomic DNA from accession G40199 revealed the presence of tightly linked genes of the complex arcelin-phytohaemagglutinin-alpha amylase inhibitor (APA) locus expressed as messenger RNA of ARL3 (accession EU-249932.1), PHApa and α-AIL (accession EU-249935.1) of P. acutifolius (Kusolwa, 2007; Kusolwa & Myers, 2009). It was unclear if this complex protein locus alone was associated with the expressions of lectin-like proteins (LLP) and if functional seed proteins for ARL, PHA and□□-AI are all confined to this locus. Identification of seed storage proteins residing in the 33 kDa subunit or any other co-segregating protein subunits is necessary in order to associate them with the expression of LLP genes observed in G40199 and its interspecific hybrids. Some or all of the APA proteins in the 33 kDa fragment may be associated with bruchid resistance as is the case of the other APA containing wild common bean genotypes (Goossens et al., 2000, Kami et al., 2006). Protein peptide identification and early expression of the seed proteins in the parent G40199 and its derived interspecific hybrid backcross lines is necessary in order to identify any modifications of gene expression between the parent and interspecific hybrids.

In G40199, it has not been clear if the genes residing in the APA locus are transcribed and expressed as functional proteins and whether post-translational alterations may occur. In addition, arcelin-like proteins in P. acutifolius have previously been described as pseudogenes, and only observed in genomic DNA (Kami et al., 2006). Earlier studies indicated that expressed mRNA sequences from these seed storage proteins might be related to translational products that constitutes the 33 kDa seed storage proteins. This fact required validation by peptide sequence analysis generated in the 33 kDa and or other homologous protein subunits in G40199. In addition, Kusolwa (2007) reported the composition of total mRNAs from the tepary bean as composed of LLP expressed in immature seeds may identify other arcelin variants. This work reports the amino acid composition, genetic expression of multiple variants of APA proteins in tepary bean G40199 and its derived interspecific backcross hybrids.

MATERIALS AND METHODS

Plant materials

Wild P. acutifolius accession G40199, and backcross inbred lines of inter-specific hybrids from the cross between ICA Pijao x G40199 as well as these backcross inbred hybrids crossed to 'Rojo' were used to study the expression and identity of the lectin-like proteins. G40199 and ICA Pijao parental materials were originally obtained from the International Center for Tropical Agriculture (CIAT), and Rojo was provided by the Sokoine University of Agriculture breeding program. A description of the development of the interspecific backcross lines can be found in Mbogo et al., (2009).

Protein extraction

The procedures of Brown et al., 1982 were followed for seed protein extraction and SDS-PAGE electrophoresis. Total seed protein, for peptide extraction, was isolated from 0.2g of ground cotyledonary bean powder and dissolved in 200 µl of extraction solution (0.5 M NaCl, 0.25 ascorbic acid pH (2.4)) and homogenized by slow and occasional vortex for 30 minutes, the mixture was left to settle at room temperature for 30 minutes then was centrifuged at 14,000 xg for 10 minutes.

SDS-PAGE seed storage protein separation

The salt soluble total seed storage proteins (10µl) were dissolved in 20µL of sample buffer containing 10% SDS, 0.5M Tris HCl pH 6.8, glycerol, 2-□-mercaptoethanol and 0.05% (w/v) Bromophenol blue. The mixture was then heated at 95oC for five minutes to denature the quaternary protein complex into linear primary structure; then cooled at room temperature before loading into a one dimension 15% Tris HCl, SDS-PAGE (BIORADTM) for electrophoretic separation of protein polypeptides at constant 150 V for 60 minutes at room temperature. Gels were stained in a 0.1% Coomasie brilliant blue R-250, 40% methanol and 10% acetic acid solution for three hours followed by 1 hour destaining in a solution of 40% methanol and 9% acetic acid to obtain clear profiles of separate protein bands. Destained gels were washed in deionized water for 1hr to remove the remnant acetic acid and methanol before isolation of the Coomasie blue stained LLP protein bands.

Isolation of peptides

The major protein band depicted at approximate 33 kDa molecular size for lectin-like proteins was carefully excised from the gel using a scalpel. Four other co-segregating protein bands in the gel

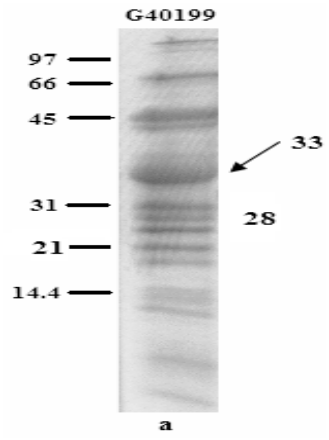


Figure 1: Protein profiles from G40199 seed (a) and backcross interspecific hybrid seed (b) as obtained from 15% Tris HCl SDS-PAGE with Coomasie blue R 250 staining. Size standards in kDa indicated to left of gels.

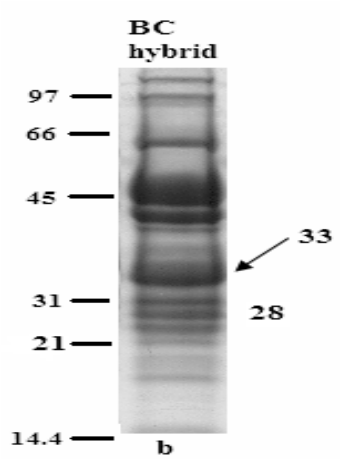


Figure 1: Cont.

profiles of approximately 31 kDa, 28 kDa, 26 kDa and 21 kDa (Figure 1) were also isolated into separate 1.5ml microfuge tubes for in-gel peptide digestion with trypsin. We hypothesized that the polypeptides in these fragments were subunits of ARCpa, ARL-2pa, □-AIpa and PHApa (Kusolwa, 2007). Each band was cut into a millimeter cubes and washed twice with 50µL of deionized water for 15 minutes followed by two destaining washes with a 50/50% solution of acetonitrile/50mM NH_4HCO_3 for 30 minutes to remove the Coomasie blue stain from the gel plugs. The gel plugs were dehydrated using acetonitrile until the gel turned opaque. Samples were dried for 30 minutes in a speed vacuum centrifuge. The gel plugs were then rehydrated with 50uL of 25mM Ammonium bicarbonate buffer containing 12.5ng/µL trypsin (Promega-Madison WI-USA) pH 8.0 for 45 minutes while chilled on ice to allow for trypsin to infuse into gel plugs. Gel plugs were then submerged in excess solution of 25mM ammonium bicarbonate to ensure proper rehydration and subjected to trypsin digestion at 37oC for six hours in the dark. Trypsin digested peptides were further sequentially extracted three times with 50% acetonitrile and the extracts were combined.

Peptide sequencing

The samples of gel digests were run by lc/MS-MS using a Waters (Millford, MA) nanoAcquity HPLC using Q-ToF-Ultima Global for separation of peptide units. One-half µl of sample was loaded onto a Waters Symmetry C18 trap at 4ul/min, and peptides were eluted from the trap onto the 10cm x 75µm Waters Atlantis analytical column at 350nl/min with solvent A - 0.1% formic acid. The HPLC gradient was 2% to 25% of solvent B (0.1% formic acid in acetonitrile) in 30 min, then to 50% solvent B in 35 min, then 80% B in 40 min and held for 5 minutes.

MS-ES data were processed with Masslynx 4.0 program to produce pkl files, a set of smoothed and centroided parent ion masses with the associated fragment ion masses. Files of parent peptide ions masses and amino acid sequences were searched with Mascot 2.0 (Matrix Science Ltd., London, UK) database software, using mass tolerances of 0.02 for the parent ion and fragment masses. The NCBInr database was used, limiting the searches to plant proteins.

RESULTS

Isolation of peptides and peptide sequencing

Expected and observed molecular sizes and amino acid sequences of peptide ions produced from SDS-polyacrylamide gel plugs excised from 33, 31, 28, 26, and 21 kDa protein subunits with their matching proteins from Mascot search were obtained and presented in chromatogram peaks. Based on significant peaks of parent peptide ions obtained during “Quadrupole-Time of Flight -Electrospray ionization” (Q-TOF-ESI) analysis, three major proteins were identified in different protein subunits. A 33 kDa size protein subunit generated a peak with peptides matching those for ARL-2pa storage proteins found in tepary beans, 31 kDa subunit fragment also matched a peptide originating from ARL-2pa and arcelin (ARCpa) proteins while the 28 kDa fragment was contained PHApa and ARCPa protein subunits. The protein fragments from the 26 kDa profile produced significant peaks for peptides that matched ARL-2pa proteins only, while the 21 kDa subunit contained peptides for ARCpa proteins (Table 1); (Figures 2 to 6)

DISCUSSION

Peptide sequencing provided revealed a matching amino acid sequences indicating the expression of

Table 1. Mascot search of observed, expected and calculated molecular sizes, amino acid sequences of peptide ions produced from excised 33, 31, 28, 26, and 21kDa protein subunits from G40199 and derived interspecific backcross hybrids are presented with their matching proteins from NCBInr database.
Score ≥ 50 indicates identity or extensive homology ($p < 0.05$).

Protein fragment	Observed	Mr. expt	Mr calc	Score	Peptide	Matched protein - reference
----------G40199----------						
33kDa	910.41	1818.81	1818.82	110	R.LTGVGSNEDPWVDSMGR.A	ARL-2 [pa] - (NCBI AF255724)
	918.44	1834.87	1834.82	79	R.LTGVGSNEDPWVDSMGR.A + Oxidation (M)	ARL-2 [pa] - (NCBI AF255724)
----------ICA Pijao x G40199 interspecific hybrid----------						
33kDa	910.41	1818.81	1818.82	110	R.LTGVGSNEDPWVDSMGR.A	ARL-2 [pa] - (NCBI AF255724)
	918.46	1834.91	1834.82	58	R.LTGVGSNEDPWVDSMGR.A + Oxidation (M)	ARL-2 [pa] - (NCBI AF255724)
----------G40199----------						
31kDa	910.40	1818.79	1818.82	118	R.LTGVGSNEDPWVDSMGR.A	ARL-2 [pa] - (NCBI AF255724)
	918.40	1834.79	1834.82	67	R.LTGVGSNEDPWVDSMGR.A + Oxidation (M)	ARL-2 [pa] - (NCBI AF255724)
	918.40	1834.79	1834.82	95	R.LTGVGSNEDPWVDSMGR.A + Oxidation (M)	ARL-2 [pa] - (NCBI AF255724)
	598.33	1194.64	1194.6	57	R.AFYSTPIQIR.D	ARC [pa] - Mirkov *et al.*, 1994
	757.39	2269.15	2269.1	72	R.ANNAGHSAYGLAFALVPVGSEPK.R	ARC [pa] - Mirkov *et al.*, 1994
28kDa	548.31	1094.61	1094.61	32	R.HIGIDVNSIK.S	PHA[pa] -Mirkov *et al.*, 1994
	900.95	1799.88	1799.90	70	R.LTNLNDNGEPTLSSLGR.A	PHA[pa] -Mirkov *et al.*, 1994
	997.53	1993.04	1993.06	47	R.VPNNAGPADGLAFALVPVGSK.P	PHA[pa] -Mirkov *et al.*, 1994
	665.36	1993.07	1993.06	23	R.VPNNAGPADGLAFALVPVGSK.P	PHA[pa] -Mirkov *et al.*, 1994
	740.41	2218.22	2218.21	69	R.VPNNAGPADGLAFALVPVGSKPK.D	PHA[pa] -Mirkov *et al.*, 1994

Table 1. Cont.

		598.32	1194.63	1194.64	46	R.AFYSTPIQIR.D	ARC [pa] -Mirkov *et al.*, 1994
		757.39	2269.16	2269.15	52	R.ANNAGHSAYGLAFALVPVGSEPK.R	ARC [pa] - Mirkov *et al.*, 1994
	26kDa	910.41	1818.81	1818.82	77	R.LTGVGSNEDPWVDSMGR.A	ARL2 [pa] - (NCBI AF255724)
		918.41	1834.80	1834.82	43	R.LTGVGSNEDPWVDSMGR.A + Oxidation (M)	ARL2 [pa] - (NCBI AF255724)
		918.41	1834.80	1834.82	8	R.LTGVGSNEDPWVDSMGR.A + Oxidation (M)	ARL2 [pa] - (NCBI AF255724)
	21kDa	640.82	1279.63	1279.63	76	K.TDVQITYESPK.K	ARC [pa] - Mirkov *et al.*, 1994

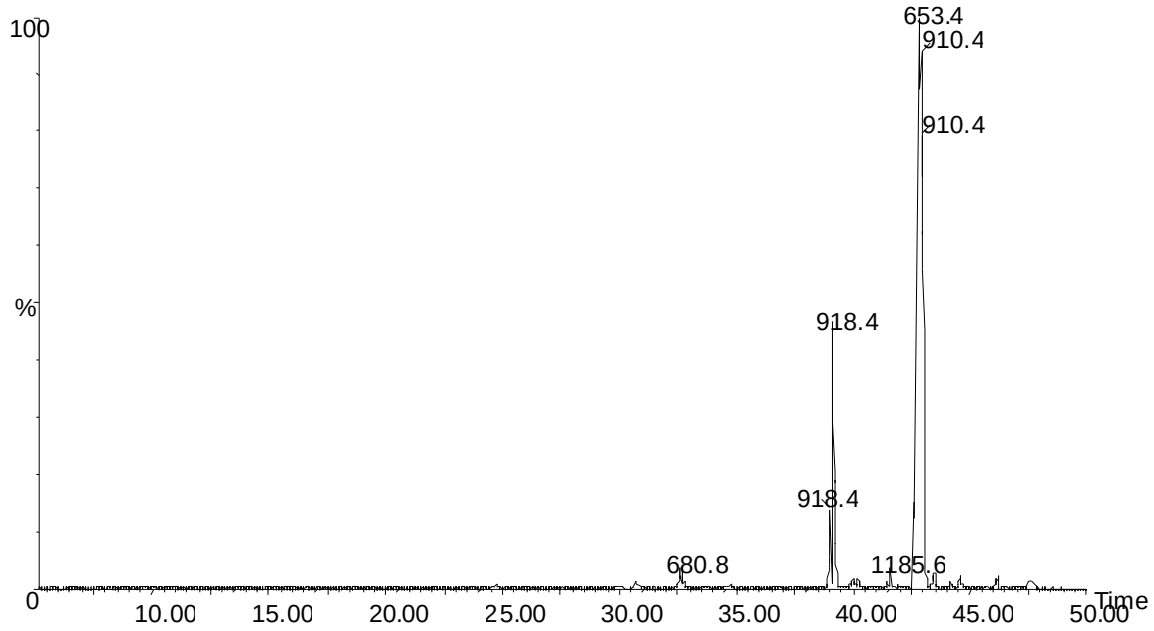
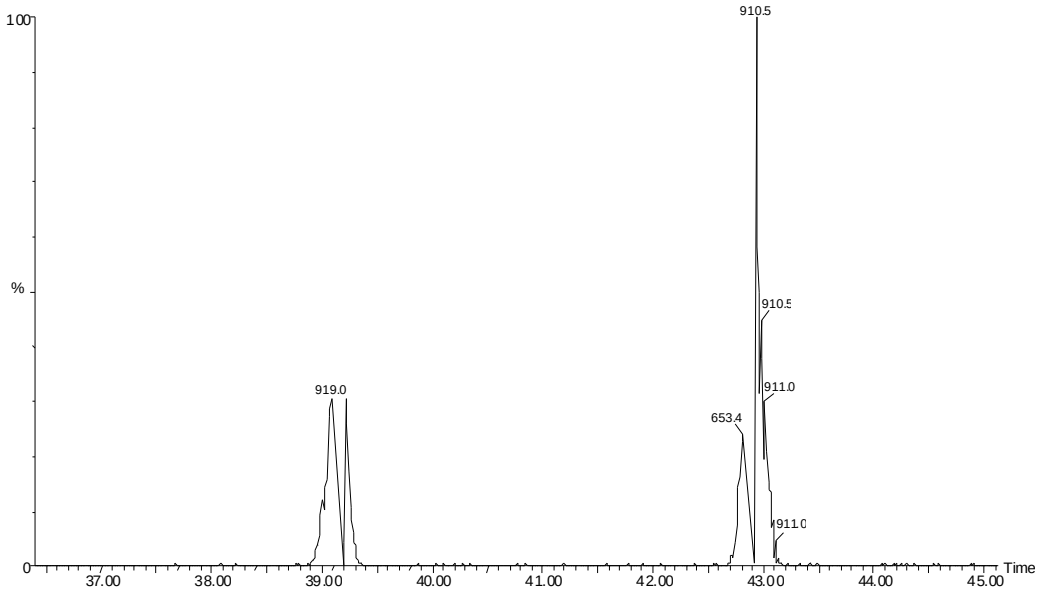


Figure 2: Chromatograms from peptide sequencing of the 33 kDa fragment in tepary bean accession G40199 indication the parent peptide ions identified by MS-MS ES analysis.



(b)

Figure 3: a and b. Chromatogram of m/z for protein peptide peaks of parent ions from 33 kDa protein produced by Q-TOF- ESI identified from seed samples obtained from interspecific hybrid ICA Pijao x G40199.

arcelins (ARL) and PHA, however, no matching peptide sequences corresponding to alpha amylase inhibitors (α-AIL) were identified from the isolated the different seed storage protein profiles isolated from SDS-PAGE plugs, one of the seed storage proteins thought to be responsible for inhibition of insect predation in tepary beans (Kami et al .,2006, Nishizawa et al., 2007). Earlier studies demonstrated similar difficulties encountered in determining the expression of the same □□-AIs in cDNA from young immature seeds of G40199 and in interspecific hybrids. Based on previous studies by Yamada et al.,(2005), Kami et al., (2006) and Nishizawa et al., (2007), we targeted a protein fragment of approximately 21 kDa for isolation of peptides that should contain alpha-amylase inhibitors fragments ions in the G40199. However, this fraction also contained matching peptides for an arcelin-like variant (ARCpa). Yamada et al., (2001, 2005) identified □-AIpa protein at a molecular size of 15-18 kDa subunits using antibodies. This implies that the estimated molecular size isolated in our experiment from the SDS gel was probably higher. In addition, we unfortunately could not isolate the faintly observed lower molecular size (14 -19 kDa) protein profile subunits from the total seed storage proteins for in-gel peptide sequencing to confirm this. Another explanation for the lack of □-AI is that the mRNA for □-AIpa in tepary beans maybe cleaved into lower molecular weight subunits, a common process in the majority of the lectin like proteins (LLPs) and leading to C-terminus truncation of mature proteins. This truncation suggests the possibility that α-AIpa was converted to another isolectin (Young et al.,1995; Lee et al., 2000). In this way, it possible that the conversion of α-AIL into an arcelin isoform, producing a concentrated quantitative expression of variants of arcelin-like proteins in the form of seed storage proteins in these tepary beans (Kusolwa & Myers 2010). In contrast to arcelin and PHA, α-AIL is synthesized as a glycosylated precursor of about 40kDa, which is proteolytically processed in the protein storage vacuoles into 15-20 kDa polypeptides of the mature inhibitor (Pueyo et al., 1993; Santino et al., 1992).

Considering the resistance to proteolytic degradation of LLP proteins, it was not possible to observe several LLP peptides in all of the seed storage protein profiles studied when compared to the theoretically predicted peptide residues that can be generated from trypsin digestion. Most of the proteins that are associated with resistance to bruchids are likely to be highly resistant to proteolytic digestion by enzymes like trypsin, chymotrypsin and pepsin. Therefore it may be impossible or rare to observe

a highly representative number of N-terminal cleavages of peptides from such proteins. In this case one may think that those proteins are absent in the total protein extract from mature bean seeds.

Besides the difficulties in detecting α-AIL proteins, peptide isolation and sequencing has clearly demonstrated that the APA locus present in accession G40199 is a complex locus that is processed into variants of active arcelin proteins in the mature seeds for protecting seeds from predation by bruchids. It will be important to determine the protein composition of lower molecular weight proteins using the same or related procedures. Increasing the amount of total protein samples for SDS-PAGE may produce higher quantity of proteins in the lower profiles. This may facilitate the isolation of lower molecular weight protease inhibitors such as trypsin inhibitors that are common components in tepary bean (Campos et al., 1997, 2004). We earlier observed a larger molecular sized protein profile that behaves as a subunit of the major 33 kDa protein because it is co-segregates in the interspecific hybrids (Kusolwa, 2007) indicating the successful transfer into cultivated species of common beans. This study has demonstrated the similar composition with the presence of arcelin like proteins and phytohaemagglutinins and the defense total seed storage proteins for bruchid predation. Further verification of the detailed identity of other parent peptides ions peaks detected in this 33 kDa fragment and other subunits is required in order to clearly validate its contribution of these proteins to bruchid resistance in common beans.

REFERENCES

Brown JWS, Osborn TC, Bliss FA, Hall TC (1982). Bean Lectins. Part 1: Relationships between agglutinating activity and electrophoretic variation in the lectin-containing G2/albumin seed proteins of French bean (Phaseolus vulgaris L.). Theor. Appl. Genet., 62: 263-271.

Campos JE, Martınez-Gallardo N, Mendiola-Olaya E, Blanco-Labra A, (1997). Purification and partial characterization of a proteinase inhibitor from tepary bean (Phaseolus acutifolius) seeds. J. Food Biochem., 21: 203-218.

Campos JE, Whitaker JR, Yip T, Hutchens TW, Blanco-Labra A (2004). Unusual structural characteristics and complete amino acid sequence of a protease inhibitor from Phaseolus acutifolius seeds. Plant Physiol. Biochem., 42: 209-214.

Cardona C, Valor JF, Mejia-Jiménez A, Beebe S, Tohme J (2005). Developing germplasm with resistance to pests: Zabrotes, Acanthoscelides - bruchids. CIAT - Ann. Rep., pp. 53-59.

Goossens A, Quitero C, Dillen W, De Rycke R, Flower Valor J, De Clercq J, Van Montagu M, Cardona C, Angenon G (2000). Analysis of bruchid resistance in the wild common bean accession G02771: No evidence for insecticidal activity of arcelin 5. J. Exp. Bot., 51: 1229-1236.

Kami J, Poncet V, Geffroy V, Gepts P (2006). Development of four phylogenetically–arrayed BAC libraries and sequence of the APA locus in Phaseolus vulgaris. Theor. Appl. Genet., 112: 987-998.

Kusolwa PM (2007). Breeding for Bruchid Resistance in Common Bean (*Phaseolus vulgaris* L.): Interspecific Introgression of Lectin-like Seed Proteins from Tepary Bean (*P. acutifolius* A. Gray), Genetic Control and Bruchid Resistance Characterization. PhD thesis, Oregon State University, 185pp.

Kusolwa PM, Myers JR (2010). Phylogenetic relationship of lectin-like proteins expressed in tepary and common beans. Aspects Appl. Biol., 96: 297-301.

Kusolwa PM, Myers JR (2011). Seed storage proteins ARL2 and its variants from the APA locus of wild tepary bean G40199 confers resistance to Acanthoscellides *obtectus* when expressed in common beans. Afr. Crop Sci. J., 19: (4) 255-265

Lee S, Gepts P, Whitaker JR (2000). Protein Structures of Common Bean (Phaseolus vulgaris) a-amylase inhibitors. J. Agric. Food Chem., 50: 6618-6627.

Lioi L, Galasso I, Santantonio M, Lanave C, Bollini R, Sparvoli F (2006). Lectin gene sequences and species relationships among cultivated legumes. Genet. Res. Crop Evol., 53: 1615-1623.

Lioi L, Sparvoli F, Bollini R (1999). Variation and genomic polymorphism of lectin-related proteins in lima bean (Phaseolus lunatus L.) seeds. Genet. Res. Crop Evol., 46: 175-182.

Lioi L, Sparvoli F, Galasso I, Lanave C, Bollini R (2003). Lectin related resistance factors against bruchids evolved through a number of duplication events. Theor. Appl. Genet., 107: 814-822.

Mbogo PK, Myers JR, Davis J (2009). Transfer of the arcelin-phytohemagglutinin – alpha amylase inhibitors seed storage proteins locus from tepary bean (P. acutifolius A. Gray) to common bean (P. vulgaris L.) Biotechnology, 8: 285-295.

Mirkov ET, Wahlstrom JM, Hagiwara K, Finardi-Filho F, Kjemtrup S, Chrispeels MJ (1994). Evolutionary relationship among proteins in the phytohaemagglutinins - arcelin and □-amylase inhibitor family of the common bean and its relatives. Plant Mol. Biol., 26: 1103-1113.

Murdock LM, Huesing JE, Nielsen SS, Pratt RC, Shade RE (1990). Biological effects of plant lectins on the cowpea weevil. Phytochemistry 29: 85-89.

NCBI (2006). National Center for Biological Information (NCBI) http://www.ncbi.nlm.nih.gov/entrez/ query.fcgi?db=protein&cmd=search&term=arcelin and http://www.ncbi.nlm.nih.gov/entrez/query.fcgi? CMD=search&DB=nucleotide

Nishizawa K, Teraishi M,•Utsumi S, Ishimoto M (2007) Assessment of the importance of □-amylase inhibitor-2 in bruchid resistance of wild common bean. Theor. Appl. Genet., 114:755-764.

Nodari RO, Tsai SM, Gilbertson RL, Gepts P (1993). Towards an integrated linkage map of common bean. 2. Development of an RFLP-based linkage map. Theor. Appl. Genet., 85: 513-520.

Pueyo JJ, Hunt DC, Chrispeels MJ (1993) Activation of bean (Phaseolus vulgaris) α-amylase inhibitor requires proteolytic processing. of the pro-protein. Plant Physiol. 101: 1341-1348.

Sales MP, Gerhardt IR, Grossi de Sá MF, Xavier-Filho J (2000). Do legume storage proteins play a role in defending seeds against bruchids? Plant Physiol., 124: 515-522.

Santino A, Daminati MG, Vitale A, Bollini R (1992). The □-amylase inhibitor of bean seed: two step proteolytic maturation in the protein storage vacuoles of the developing cotyledon. Physiol. Plant, 85: 425-432.

Sparvoli F, Gallo A, Marinelli D, Santucci A, Bollini R (1998). Novel lectin-related proteins are major components in lima bean (Phaseolus lunatus) seeds. Biochim. Biophys. Acta, 1382: 311-323.

Sparvoli F, Lanave C, Santucci A, Bollini R, Lioi L (2001). Lectin and lectin-related proteins in lima bean (Phaseolus lunatus L.) seeds: biochemical and evolutionary studies. Plant Mol. Biol., 45: 587-597.

Van Damme EJM, Peumans WJ, Barre A, Rougé P (1998). Plant lectins: A composite of several families of structurally and evolutionary related proteins with diverse biological roles. Crit. Rev. Plant Sci., 17: 575-692.

Yamada T, Hattroi K, Ishimoto M (2001). Purification and characterization of two α-amylase inhibitors from seeds of tepary bean (Phaseolus acutifolius A. Gray). Phytochemistry, 58: 59-66.

Yamada T, Moriyama R, Hattori K, Ishimoto M (2005). Isolation of two α-amylase inhibitor genes of tepary bean (Phaseolus acutifolius A. Gray) and their functional characterization in genetically engineered adzuki bean. Plant Sci., 169: 502-511.

Young MN, Watson DC, Yaguchi M, Adar R, Arangos R, Rodriguez-Arangos E, Sharon N, Blay PKS, Thibault P (1995). C-terminal post-translational proteolysis of plant lectins and their recombinant forms expressed in Escherichia coli: Characterization of ragged ends by mass spectrometry. J. Biol. Chem., 270: 2563-2570.