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Arcelins from an Indian Wild Pulse, *Lablab purpureus*, and Insecticidal Activity in Storage Pests

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A partially purified protein fraction was isolated from seed flour of the Indian wild bean, *Lablab purpureus*, by ion exchange and size-exclusion chromatographies. Partially purified *L. purpureus* proteins had hemagglutination and glycoslyation properties similar to those of lectins or lectin-like proteins from other pulses. Data obtained from two-dimensional gel electrophoresis, MALDI-TOF, and MALDI-TOF/TOF and N-terminal protein sequencing of the isolated polypeptides from *L. purpureus* demonstrated that the extract contained proteins similar to isoforms of arcelins 3 and 4 and pathogenesis-related protein 1 (PvPR1) of *Phaseolus vulgaris*. *L. purpureus* proteins were resistant to degradation by the commercial enzymes trypsin and chymotrypsin and were moderately resistant to pepsin, but were readily hydrolyzed to smaller peptides by papain. Insect feeding bioassays of the extract with the storage pests *Rhyzopertha dominica* and *Oryzaephilus surinamensis*, internal and external feeders of grain, respectively, demonstrated that *L. purpureus* proteins at 2% in the diet resulted in retarded development. However, a 5% dose of the *L. purpureus* fraction resulted in complete mortality of all larvae in both species. This study has demonstrated that proteins in the partially purified *L. purpureus* extract have the potential to control storage pests in cereals transformed with *L. purpureus* defense-related genes, but the need for more studies regarding efficacy and safety is discussed.

KEYWORDS: Arcelin; *Phaseolus* spp; insecticidal proteins; bruchids

INTRODUCTION

The storage of agricultural products is problematic due to physicochemical parameters and damage by biological agents, primarily insects. Pulses in storage are protected from pest damage through the control of temperature, humidity, or the introduction of volatiles, such as methyl bromide, phosphine, and sulfur (*1–3*). Despite all of these measures, insect fauna, such as *Callosobruchus maculatus* and *C. chinensis*, successfully colonize and inflict severe damage to stored beans. In this context, the identification and manipulation of inherent resistance mechanisms, such as antibiosis in cultivated crop varieties and their wild relatives, provide an effective method to manage stored-product pests. The exploitation of natural resistance mechanisms against insect pests is the most accepted alternative to synthetic pesticides in the recent strategies of integrated pest management (IPM) programs.

Many cultivated bean varieties with good yield potentials are highly susceptible to stored-product insect pests, whereas often wild varieties of pulses have developed chemical defenses against insects by accumulating toxic compounds, such as phenolics, cyanogens, tannins, protease inhibitors, amylase inhibitors, and lectins. The evidence suggests that legumes have adapted to insect predators primarily through the expression of insecticidal lectins and related proteins, although there is ambiguity in the insecticidal components and mode of action.

Among the insect defense compounds in wild pulse accessions, those with insecticidal potential include the lectin-like storage proteins, arcelins (*4, 5*). Seven allelic variants of arcelin have been described in the genus *Phaseolus* (*6–10*). Arcelins copurify with the lectin phytohemagglutinin and possibly other lectin-like proteins (*4*), a fact that may have contributed to confusion in the interpretation of insect bioassays. Phytohemagglutinin from *Phaseolus vulgaris* appeared to be an effective insecticide in bruchid beetles (*11*). However, it was the *P. vulgaris* lectin-like inhibitor of α-amylase, and not phytohemagglutinin, that was later determined to be toxic to *C. maculates* (*12*), although the α-amylase inhibitor had no activity in other bruchids, such as *Acanthoscelides obtectus* and *Zabrotes subfasciatus*. In contrast, arcelin variants were highly toxic to *Z. subfasciatus*, but not to other bruchids, such as *A. obtectus* (*13*).

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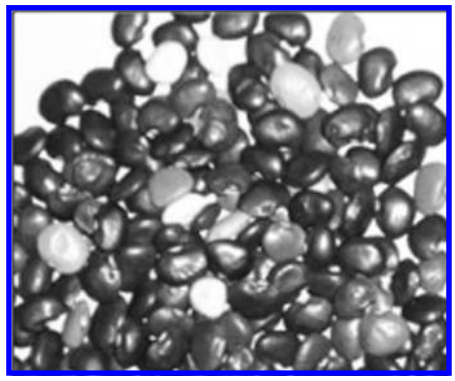

Figure 1. Seeds collected from wild pulses of *L. purpureus*.

The mechanism of arcelin toxicity in insects is speculative. Paes et al. (*14*) proposed that the insecticidal activity of areclin-1 was due to the disruption of the epithelial cells lining the gut. Others hypothesized that toxicity mediated by arcelin was caused by the interaction of the protein with specific glycosylation in the gut (*15, 16*) or by providing the insect with a source of poorly digestible protein (*17*).

The problem with conventional physicochemical methods that have been used to protect beans in storage is the reduction in quality, sometimes resulting in a product that is unsuitable for consumption. The incorporation of inherent plant protection strategies can provide an environmentally safe and more targeted alternative to pest control. Protection against damage by storage pests may be achieved through the engineering of genes that encode insecticidal proteins from a non-native cereal or other plant, such as wild pulses. Transgenic bean crops with synthetic arcelin genes already have been developed (*18, 19*).

To identify new genes to include in the transgenic arsenal, we have studied proteins isolated from the wild Indian pulse *Lablab purpureus*. The first report of antibiosis in this legume detailed inhibitors of α-amylase that reduced the damage of the fungal pathogen *Aspergillus flavus* (*20*). In a preliminary study, a semipurified fraction from a crude extract of *L. purpureus* seeds was insecticidal to the bruchid *C. maculatus* (*21*). This paper characterizes proteins in this *L. purpueus* extract and evaluates the effect of the extract on an internal- and external-feeding storage pest.

MATERIALS AND METHODS

Purification of *L. purpureus* Arcelin and Related Proteins. Seeds from a wild germplasm of the pulse variety *L. purpureus* (**Figure 1**) were used for extraction according to a procedure adapted from Osborn et al. (*4*). Briefly, after removal of the seed coat, the seeds were ground, and protein was extracted from 10 g of finely powdered seed flour in 100 mL of 25 mM sodium acetate buffer, pH 4.6 (buffer A), stirred overnight at 4 °C. The extract was subjected to centrifugation at 15000*g* for 25 min at 4 °C. The supernatant was filtered through cotton wool to remove particulates.

Extracted proteins were purified by ion exchange chromatography on CM-cellulose (*17*). A 40 × 1.6 cm diameter, 80 mL bed volume column of CM-cellulose (Pharmacia, Kalamazoo, MI) was equilibrated with buffer A at a flow rate of 10 mL/min. A total of 50 mL of filtered supernatant was loaded onto the column and, after adsorption, the column was washed overnight with buffer A. Bound material was eluted with a sodium chloride gradient (0.0−0.5 M) in buffer A. Fractions containing *L. purpureus* arcelins and associated proteins (Lp arcelins) eluted between 0.18 and 0.24 M NaCl and were detected in a single peak at 280 nm. Fractions were pooled and dialyzed in buffer A overnight, and protein was precipitated with 90% (w/v) ammonium sulfate. The precipitate was dissolved in 10 mL of 25 mM sodium acetate, pH 7.2, and proteins were analyzed by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) (*22*) using 4–12% Bis-Tris gels with MES buffer (Invitrogen, Carlsbad, CA).

The dissolved ammonium sulfate-precipitated proteins were dialyzed against distilled water and lyophilized. Lyophilized proteins were resuspended in 0.1 M sodium borate buffer (pH 7.0, 0.1 M NaCl) and were further purified by gel filtration chromatography using Sephacryl S-400 (1.6 × 40 cm column, Pharmacia). The elution profile was compared to a profile from an identical column calibrated with protein standards. Fractions were analyzed by non-denaturing PAGE (4–20% Novex Tris−glycine gels with Tris−glycine buffer), SDS-PAGE (4–12% Novex Bis-Tris gels with MES buffer, Invitrogen), and two-dimensional (2-D) electrophoresis, using IEF (Novex IEF gels, pH 3–10) for the first dimension and 4–12% Bis-Tris SDS-PAGE (Zoom gels) for the second dimension (all gels and buffers were from Invitrogen). Gels were stained with Coomassie R-250 (Imperial Protein Stain, Pierce Chemical Co., Rockford, IL).

For protein transfer to PVDF, after electrophoresis, the gel was rinsed in transfer buffer (NuPAGE, Invitrogen) for 5 min. Proteins were electroblotted to polyvinylidene difluoride (PVDF, Immobilon PSQ, Millipore, Billerica, MA) at 30 V for 1 h. After protein transfer, the PVDF was rinsed 3 × 5 min in deionized water. Proteins were detected by staining with Coomassie brilliant blue R-250 (0.1% in 50% methanol) for 5 min and destaining with several changes of 50% methanol−10% acetic acid. The membrane was rinsed in several changes of deionized water, and protein spots were excised for N-terminal sequencing.

Physicochemical Measurements. Analysis of the total neutral sugar content of the proteins in the arcelin fractions was determined according to the phenol−sulfuric acid method of Dubois et al. (*23*) using glucose as standard. Proteins in the extract also were tested for agglutination of rabbit red blood cells (RBC) using a modified procedure described previously (*4*). Briefly, glutaraldehyde-stabilized RBC were washed three times in 10 mM PBS, pH 7.4 (Sigma Chemical Co., St. Louis, MO). The cells were resuspended in PBS at 1% concentration for the assay, performed in a 96-well flat-bottom microtiter plate. Purified Lp arcelin was dissolved in PBS at a concentration of 3 mg/mL and diluted serially 1:1 in PBS. To the protein dilutions, 10 μL of 1% RBC was mixed and incubated for 2 h at room temperature with gentle rocking. To evaluate the effect of enzyme treatment or heat denaturation, Lp arcelin was incubated with papain (see Enzyme Assays for details) or was preheated at 95 °C for 5 min prior to the addition of RBC. A negative control was maintained by mixing 10 μL of RBC with 25 μL of PBS or papain. The plate was read visually by observing precipitate in the well.

MALDI-TOF and N-Terminal Sequencing. For sequence analysis, gel plugs from a Coomassie-stained 2-D gel were excised and digested with trypsin (Trypsin Gold, sequencing grade porcine, Promega, Madison WI), using a standard proteomic digest protocol (*24*). Peptides in the digests were analyzed by MALDI-TOF and MALDI-TOF/TOF using Mascot software and searching the database NCBI-nr (UltraFlex II, Bruker Daltonics, Billerica, MA; Matrix Science, Boston, MA). N-Terminal sequencing was from a sample transferred to PVDF and analyzed by reverse-phase HPLC using automated Edman chemistry (Procise 492 Protein Sequencer, Applied Biosystems, Foster City, CA). All sequencing and mass spectra analyses were performed at the Kansas State University Biotechnology Core Laboratory.

Enzyme Assays. To check the resistance of arcelin to hydrolysis by enzymes from purified commercial sources, Lp arcelin was digested under buffer conditions appropriate for each enzyme with equimolar amounts of commercial enzymes. Optimal reaction buffers and conditions for each enzyme were according to those of Sarath et al. (*25*) or were those recommended by the manufacturer.

Trypsin and Chymotrypsin. The Lp arcelin fraction was dissolved in 100 mM ammonium bicarbonate, pH 7.8, at a concentration of 1 mg/mL. Trypsin (type IX, porcine pancreas, Sigma) and chymotrypsin (DFP treated, Worthington Biochemical, Freehold, NJ) were dissolved in 50 mM Tris, pH 8.0, and 10 mM $CaCl_2$. The enzymes were added to arcelin at a ratio of 5:50 (w/w) and incubated for 4 h at 37 °C.

Pepsin. The Lp arcelin fraction was dissolved in 10 mM HCl at a concentration of 1 mg/mL, and the pH was adjusted to 4.0 using a saturated NaOH solution. Pepsin (1 mg/mL; Worthington) was dissolved

in 4 M urea, and 3 M guanidine−HCl and was added to arcelin at a ratio of 5:50 (w/w) and incubated for 1 h at 25 °C.

Papain. The Lp arcelin fraction was dissolved in water at a concentration of 1 mg/mL. Papain (1 mg/mL; papaya latex, Sigma) was dissolved in sodium phosphate buffer, pH 7.0, with 5 mM L-cysteine and was added to Lp arcelin at a ratio of 5:50 and incubated for 1 h at 25 °C.

For controls, the substrate (Lp arcelins) or enzymes in respective buffers were used as positive control (without any treatment) and negative control (corresponding to each enzyme time and temperature). All hydrolysates were separated by 10% Tris-Bis SDS-PAGE and MES buffer (Invitrogen) and were stained by Commassie blue to evaluate reaction products.

Insect Bioassay. Lp arcelins were bioassayed at 2 and 5% of the test diet with internal- and external-feeding pest species of cereal grain: the lesser grain borer, *Rhyzopertha dominica* (Fabricius), and the sawtoothed grain beetle, *Oryzaephilus surinamensis* (Linnaeus), respectively. After dialysis against water and lyophilization, partially purified Lp arcelin powder was ground in a mortar with a control diet consisting of 5% brewer's yeast (Sigma) and 95% whole wheat flour. Each egg of *R. dominica* was placed on 8 mg of diet in a 0.2 mL capillary pipet tip (after the bottom of the tip had been sealed by twisting off the capillary portion), whereas each egg of *O. surinamensis* was placed on 6 mg of diet in a 0.2 mL PCR microcentrifuge tube (after three holes had been made in the lid to allow air circulation), a total of 15 to 18 eggs for each species. The diet was pre-equilibrated at the bioassay conditions of 28 °C, constant darkness, and ~75% relative humidity (over a saturated solution of sodium chloride). Containers with unhatched eggs were discarded, and larval mortality was recorded as well as the developmental time from egg hatch to adult eclosion and, in the case of *O. surinamensis*, the adult sex and weight. Statistical tests used to evaluate differences included the Fisher exact test and the Tukey test.

RESULTS

Purification of Protiens from *L. purpureus* Seed Extract. In this study, seed flour from *L. purpureus* was used as a source to obtain proteins with homology to arcelins from other pulses. From 15 mL of soluble flour extract, ion exchange chromatography resulted in 46 pooled fractions eluting within the NaCl gradient of 0.18−0.22 M, with a sample yield of 200 μg/mL total protein (9.2 mg total). Two major groups of proteins were detected in the partially purified fractions, with molecular masses of approximately 32 and 36 kDa (**Figure 2**, lane 3). A native protein with an apparent molecular mass of 140 kDa was detected in the gel filtration-purified sample, according to the elution profile compared to standards, and was a single band on a native gel (data not shown).

Proteins in the sample were focused to distinct bands between p*I* 5.3 and 6.0 in an isoelectric focusing gel (**Figure 3A**). At least two major and two minor protein bands were observed. The second-dimension separation of these proteins by SDS-PAGE indicated that there were at least two major groups of proteins and several minor proteins in the purified Lp arcelin fraction (**Figure 3B**). The molecular masses of these proteins were similar to those observed by SDS-PAGE (compare to **Figure 2**).

Mass Analysis of Lp Arcelins. Coomassie-stained Lp proteins were excised from the 2-D gel (**Figure 3B**), gel plugs were digested by trypsin, and the peptide mass fingerprint of the resultant peptides was analyzed by MALDI-TOF. Fragments from fractions 1 and 2 of the 2-D gel were similar to those of *P. vulgaris* arcelins, and the mass analysis of fragments from digests of fraction 2 resulted in 89.2% coverage of the arcelin variant 3 isoform (data not shown). Two fragments from the digests of fraction 2, with *m*/*z* ratios of 1776 and 2019.86, were analyzed by MALDI-TOF/TOF in the "lift" mode to obtain

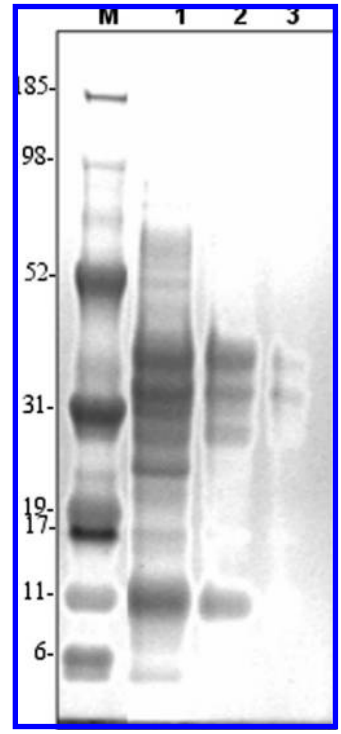

Figure 2. Coomassie blue stained gel of *L. purpureus* proteins separated by SDS-PAGE: lane 1, crude extract; lane 2, pooled and dialyzed fractions from ion exchange chromatography; lane 3, proteins precipitated with ammonium sulfate; lane M, molecular mass markers.

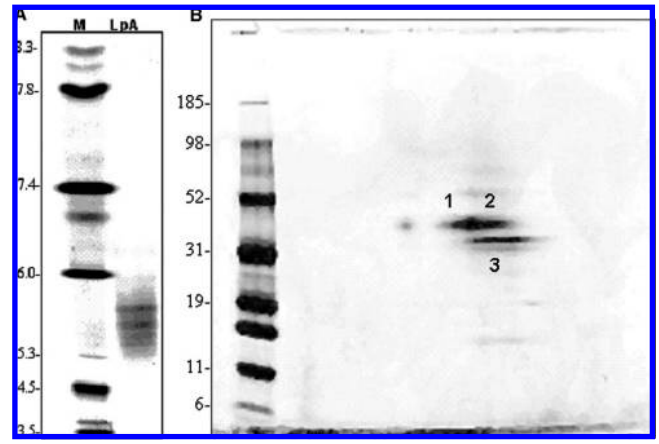

Figure 3. Two-dimensional separation of arcelins from *L. purpureus* (Lp): (**A**) isoelectric focusing of chromatographic fraction containing Lp arcelin (lane M, isoelectric markers); (**B**) second-dimension separation of isoelectric-focused fraction; molecular mass markers are indicated on the left. Arrows and numbers refer to the fractions that were trypsinized and analyzed by MALDI-TOF and MALDI-TOF/TOF and/or were subjected to N-terminal sequencing.

sequence information. Partial peptide sequences of these fragments were **C L S V D V L S W S F**, aligning to highly conserved regions in the C terminus of arcelin variants 3–5 and 7, and **W V N L G F S AC C**, with some similarity to most of the arcelin variants (**Figure 4**). The N-terminal sequence of a protein in fraction 1 of the Lp arcelins was **A S E T S**, most similar to sequences found in the N-terminal region of arcelin 3 and 4 isoforms of *P. vulgaris*. Taken together, these fractions in the Lp arcelin extract were similar to *P. vulgaris* arcelin isoforms 3 and 4.

Attempts to analyze fragments of fraction 3 did not provide significant Mascot scores. However, N-terminal analysis of

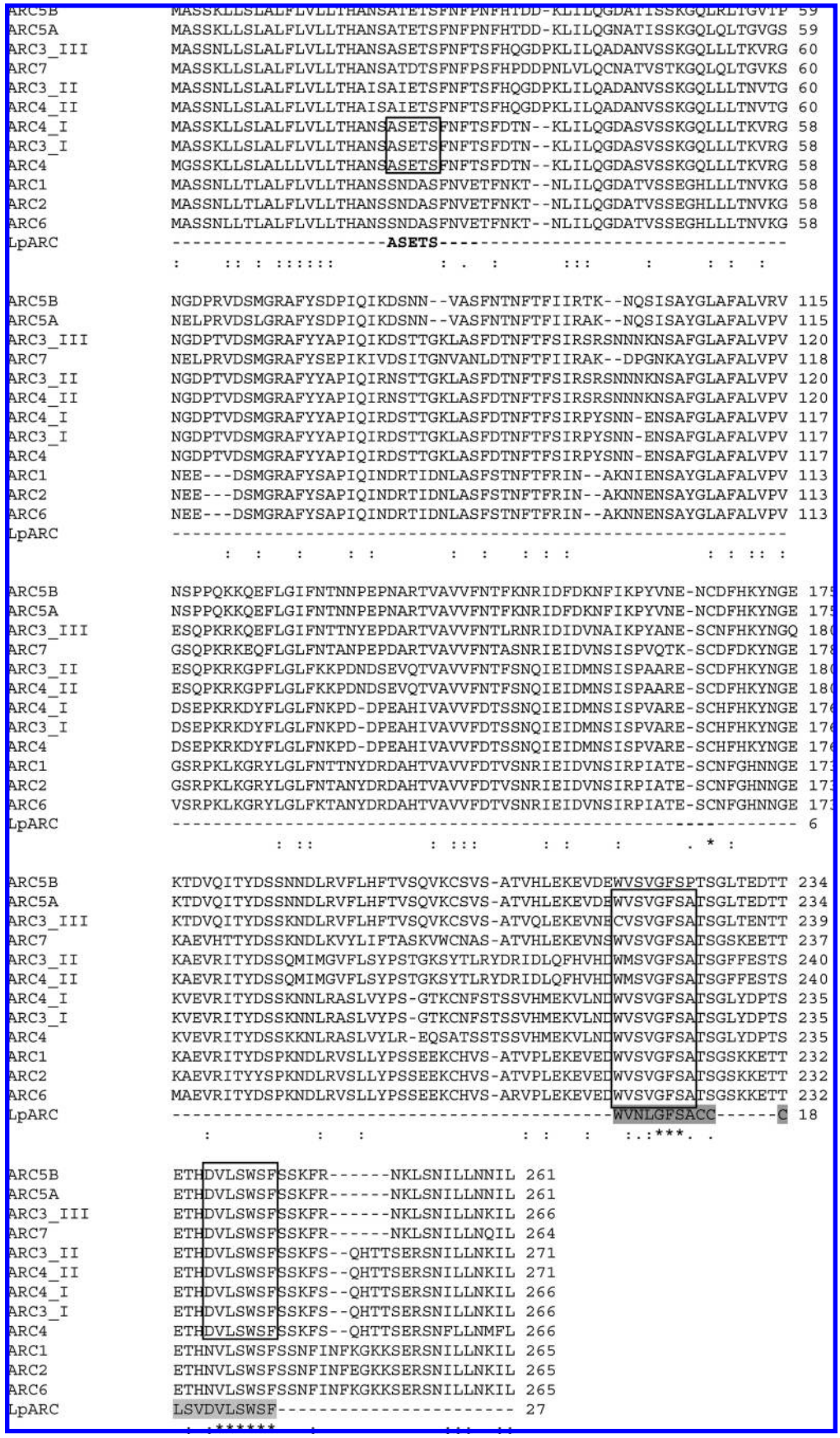

Figure 4. Clustal alignment of *P. vulgarus* arcelin isoforms with MALDI-TOF/TOF sequences of trypsin-digested peptides from *L. purpureus* arcelin (LpARC, shaded) and the N-terminal of Lp arcelin (boldface). Conserved regions are boxed. Accession numbers are as follows: ARC1, P19329; ARC2, P19330; ARC3_I, CAD58972; ARC3_II, CAD27954; ARC3_III, CAD58657; ARC4, Q43629; ARC4_I, CAD29134; ARC4_II, CAD58679; ARC5A, Q42460; ARC5B, Q41116; ARC6, CAA04960; ARC7, CAD28677 (*5, 9, 10, 34–36*). (*) are conserved residues; (:) are conserved substitutions; (.) are semiconserved substitutions.

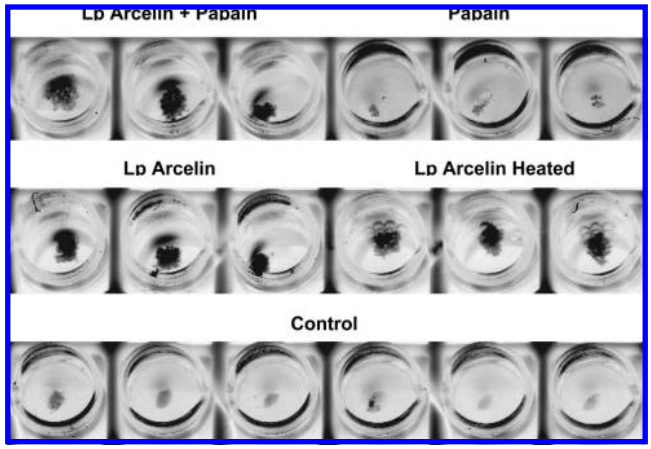

Figure 5. Effect of papain (Lp Arcelin + papain) or heat denaturation (Lp Arcelin Heated) on the hemeagglutination properties of *L. purpureus* (Lp) arcelin with RBC, compared to Lp arcelin alone (Lp Arcelin), papain only (Papain), or RBC only (Control). Each treatment is in triplicate, except for the control ($n = 6$).

fraction 3 resulted in the sequence **G I F S F E E Q T F**, which has identity (66%) with the N terminus of pathogenesis-related protein 1 (PvPR1) of *P. vulgaris* (accession no. gi:130829 (*26*),). This protein has been reported as up-regulated in bean leaf response to fungal infection.

Physicochemical Assays. *Phaseolus* spp. arcelins have strong agglutinating activity with Pronase-treated rabbit, mouse, or human erythrocytes (*4, 15*). Therefore, LP arcelin was tested for hemeagglutinating activity with rabbit RBC. Haemagglutination was observed in incubations of RBC (no enzyme treatment) containing >16 μg/mL of LP arcelin (data not shown), similar to the value of 8.2 μg/mL that previously was reported for *P. vulgaris* arcelins and erythrocytes (*27*). The agglutination potential of Lp arcelins was not affected by enzyme treatment (**Figure 5**, arcelin + papain) or by heat denaturation (**Figure 5**, arcelin heated). The neutral sugar content of the purified Lp arcelins was 16.94% (w/w). This was within the range of the percent glycosylation previously reported for *P. vulgaris* arcelins, from 16.50 to 20.05% (*4*).

Digestion by Enzymes. One of the mechanisms proposed for the insecticidal activity of arcelin is the indigestibility of arcelins in the insect gut (*17*). To evaluate Lp arcelin in this respect, the ability of commercial enzymes to hydrolyze proteins in the Lp arcelin fraction was tested. Lp arcelins were resistant to trypsin and chymotrypsin, as minor hydrolysis was observed after 4 h of incubation (**Figure 6A**, lanes 3 and 4, respectively). Incubations with pepsin under acidic conditions indicated that Lp arcelins were moderately resistant to pepsin, especially the slower migrating proteins in the fraction (**Figure 6B**, lane 2). However, the Lp arcelin fraction was almost completely degraded by papain (**Figure 6B**, lane 6).

Insect Bioassay. Although there is evidence that arcelins are toxic to certain bruchids (*13*), these proteins have not been tested extensively against storage pests. Therefore, Lp arcelins were tested in bioassays for their effect on the growth and development, as well as mortality, of the feeding stages of two stored-product insect species. The development from egg to adult was significantly delayed in both *R. dominica* and *O. surinamensis* when fed diets containing Lp arcelins (**Tables 1** and **2**), respectively. Lp arcelins added to the diet at 5% resulted in 100% mortality of larvae from both species. In the bioassay with *O. surinamensis*, the mortality was increased and the mean weight of adult males was decreased on diets containing 2% arcelin compared to those on control diets

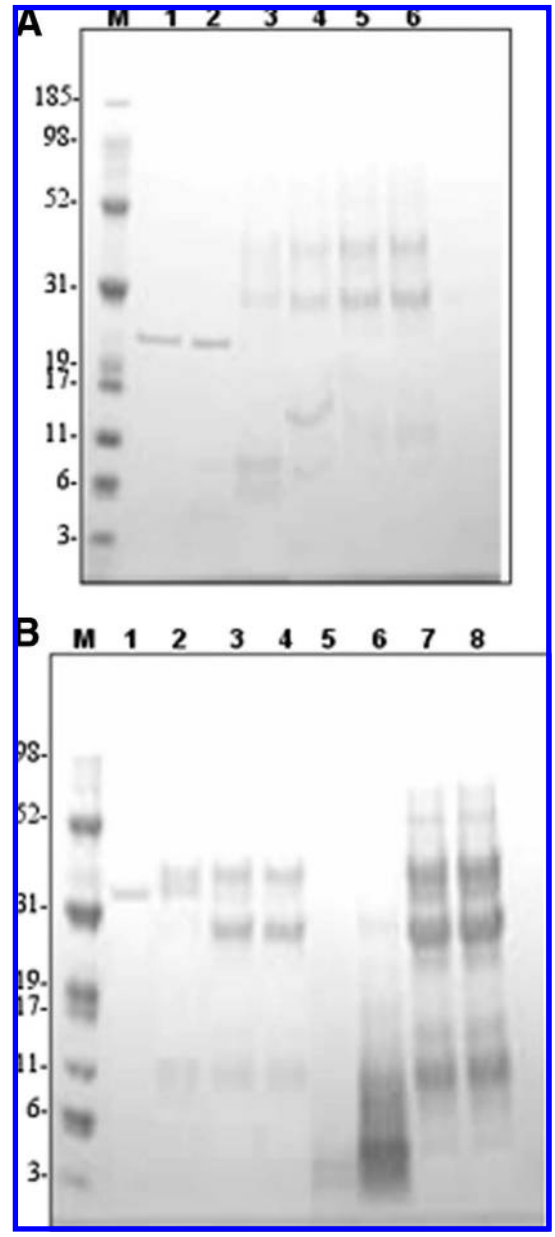

Figure 6. Enzyme stability of *L. purpureus* (Lp) arcelins: (**A**) treatment of LP arcelin with trypsin and chymotrypsin [lane 1, trypsin only; lane 2, chymotrypsin only; lane 3, trypsin + Lp arcelin; lane 4, chymotrypsin + Lp arcelin; lane 5, Lp arcelin preincubation; lane 6, Lp arcelin postincubation (no enzyme control)]; (**B**) treatment of Lp arcelin with pepsin and papain [lane 1, pepsin; lane 2, pepsin + Lp arcelin; lane 3, Lp arcelin preincubation; lane 4, Lp arcelin postincubation (in 10 mM HCl, no enzyme control); lane 5, papain; lane 6, papain + Lp arcelin; lane 7, Lp arcelin preincubation; lane 8, Lp arcelin postincubation (buffer only, no enzyme control).

(**Table 2**). A preliminary assay of the 2% Lp arcelin preparation with an additional species, the maize weevil (*Sitophilus zeamais* Motschulsky) indicated that immature development within artificial seeds was delayed (data not shown). These results demonstrate that the 2% Lp arcelin preparation delayed development of *R. dominica* and *O. surinamensis*, whereas the 5% concentration was highly toxic.

DISCUSSION

Previously, a semipurified arcelin fraction from *L. purpureus* seeds was found to be insecticidal to *C. maculatus* at concentra-

Table 1. Effect of Partially Purified *L. purpureus* (Lp) Arcelins on *R. dominica*

diet	mortality (%)	development[a] (days)
control	13	27.2 ± 0.4 (13)
2% Lp arcelin	20	54.0 ± 2.1 (12)[b]
5% Lp arcelin	100[b]	− (18)

[a] Data for development from egg hatch to adult eclosion are mean ± SE, with the number of insects in parentheses. [b] Significantly different from the control at the 0.05 level, according to the Fisher exact test (for mortality data) or the Tukey test (for development data).

Table 2. Effect of Partially Purified *L. purpureus* (Lp) Arcelins on *O. surinamensis*

diet	mortality (%)	development[a] (days)	adult wt[a] (μg)	
			female	male
control	0	21.3 ± 0.4 (12)	563 ± 25 (7)	488 ± 29 (5)
2% Lp arcelin	38[b]	32.1 ± 0.8 (8)[b]	477 ± 63 (2)	364 ± 26 (6)[b]
5% Lp arcelin	100[b]	− (13)		

[a] Data for development from egg hatch to adult eclosion and for adult weight are mean ± SE, with the number of insects in parentheses. [b] Significantly different from the control at the 0.05 level, according to the Fisher exact test (for mortality data) or the Tukey test (for development data).

tions >0.06% (w/w) in artificial seeds, resulting in 100% mortality (*21*). To provide a more thorough analysis of arcelins from *L. purpureus*, this paper details biochemical analyses of a partially purified extract from *L. purpureus* seed flour.

Arcelins from wild accessions of *Phaseolus* spp. have subunits of approximately 27400 to 35800 Da and are glycosylated (*4*). A native form of *P. vulgaris* arcelin has a molecular mass of 159600 Da, and arcelin isoforms have isoelectric points of 6.7–6.8. Proteins in the Lp extract were similar in mass to arcelin isoforms in other pulses, but the molecular mass of the native Lp arcelin was slightly less and the isoelectric points of isoforms were more acidic than those of other pulse arcelins. Mass and sequence analysis indicated that trypsin-digested proteins in the Lp extract were similar to those of *P. vulgaris* arcelin isoforms 3 and 4 and, possibly, PvPR1. Therefore, this purified extract of *L. purpureus* proteins contained multiple isoforms of arcelins and potentially other plant defense proteins. Genes encoding arcelins 3 and 4 cluster together in a phylogenetic analysis and were determined to be the earliest ancestors of the arcelin gene lineage (*10*). The physicochemical characteristics, including the ability of arcelins to agglutinate rabbit RBC and glycosylation containing neutral sugars, also were similar to those of other pulse arcelins. However, the finding that the agglutination property of proteins in the Lp arcelin extract is refractory to enzyme hydrolysis and heat denaturation may be problematic in the incorporation of arcelin into transgenic cereals, if in fact these properties belong to the arcelin proteins.

The proteins in the Lp arcelin extract were highly resistant to degradation by trypsin and chymotrypsin and somewhat resistant to pepsin, but were almost completely degraded by papain. One of the theories for the selectivity of arcelin toxicity is the lack of digestibility in the insect gut, essentially starving the insect of required amino acids (*17*). In that study, *Phaseolus* arcelin 4 was resistant to proteolysis by unidentified larval gut enzymes from *Z. subfaciatus*, an insect that is highly susceptible to the toxin (*13*). Alternatively, a cysteine protease was purified from the gut of an insect resistant to arcelins, *A. obtectus* (*28*). Consistent with the hypothesis that insects with digestive cysteine proteases are protected from arcelin toxicity, we found that the cysteine protease papain can almost completely degrade Lp arcelins. However, *C. maculatus* also has cysteine proteases for protein digestion (*29–31*), and yet it is susceptible to Lp arcelins (*21*). The contradiction argues against the starvation mode of action, but an alternative explanation is that the Lp arcelin extract has proteins other than arcelins that are toxic to *C. maculatus*, such as, for example, α-amylase inhibitor. Although the Lp arcelin fraction may contain PvPR1, the insecticidal properties of this protein are unknown.

Prior to this study, only certain bruchids were reported to be affected by arcelins. The amount of *P. vulgaris* arcelin to induce mortality in *Z. subfaciatus* was 10% of the diet (*5*), much greater than the 0.06% Lp arcelin concentration in artificial seeds that resulted in 100% mortality of *C. maculatus* (*21*) or the 5% levels used in this study. It is not known whether the difference in efficacy is due to the protein source and/or the insects. Nonetheless, this paper is the first of the expanded host range of arcelins in storage pests, including the internal feeder *R. dominica* and the external feeder *O. surinamensis*. Although the literature is scant, serine proteases appear to be the predominant digestive enzymes for protein hydrolysis in these storage pests ((*32, 33*); B. Oppert, unpublished data). However, as previously noted, more work is needed to correlate digestive enzymes and arcelin toxicity.

As cereal producers lose current control products to deregulation and insect resistance, there is a critical need to identify new insecticidal proteins for the control of storage pests. Bruchid damage to stored beans is more problematic in East Africa and other developing countries, especially where longer storage times are required. The successful isolation of genes encoding novel insecticidal proteins provides new material for the development of cereal and pulse varieties with enhanced protection to storage pests. In fact, beans have already been transformed with arcelin genes and have high levels of transgenic protein (*18, 19*). However, the efficacy of these transgenics against bruchids has not been impressive, and more research is needed to determine if other plant factors are needed for coexpression. Certainly, the genes encoding *Bacillus thuringiensis* toxins have demonstrated efficacy in transgenic crops against several target pests, at levels much lower than those in this study. However, *B. thuringeinsis* toxins have low efficacy in the control of many coleopteran storage pests.

More research is needed regarding the mode of action of arcelins in insects and mammals prior to the development of transgenic cereals. The analysis of Lp arcelins in this study suggests that there are several other proteins in the Lp arcelin fraction. All proteins that copurify with pulse arcelins need to be evaluated for insect and mammalian toxicity before the progression to crop or cereal transformation. In addition, the host range and potency of Lp arcelins in storage pests can help to determine their role in IPM strategies.

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