Arcelins – A Potential New Age Protein Anti-metabolite in Legume Seed Defense Against Stored Product Pests

S Janarthanan*, S Seshadri and S Ignacimuthu**

Entomology Research Institute, Loyola College, Chennai 600 034, India
*PG and Research Department of Zoology, Thiagarajar College, Madurai 625 009, India

Worldover there is a search for newer protein anti-metabolites from plants for insect pest control. A range of proteins in this line has been identified and discussed by various people for more than three decades. Use of protein anti-metabolites in plant transformation is a very recent concept in insect pest control. Arcelins, the abundant seed storage proteins from wild varieties of pulses offer promising results against stored product insect pests. Though identified during late 80s, arcelins are studied for effective exploitation at mass level to confer resistance to seeds under storage. This paper discusses about arcelins, their molecular characteristics, toxic nature to insect pests, and possibilities for further exploitation in insect pest control.

Introduction

Bruchids are the most serious insect pests attacking food legumes during storage. Some are generalists, exploiting many species of legume seeds, while others appear to be specialists having a restricted range of hosts. Many of the bruchid genera feed on seeds from more than one legume subfamily. Thus, 50 per cent of the 56-bruchid genera are able to develop on the Caesalpinioidea, 56.2 per cent on Mimosoidea and 59.3 per cent on Papilionoidea. In addition, 25 per cent of the bruchid genera have been recorded from all three subfamilies of legumes. Factors reported to control the insect attack are seed suitability, tissue hardness and thickness, surface chemicals, toxic internal constituents, specific defense compounds, insect enzyme inhibitors, etc. The past three decades have seen an increased awareness among world scientists in elucidating the mechanism of seed resistance to insect attack.

Self Defense by Plants

During the course of evolution, plants have developed several chemical defenses against herbivore insects by accumulating toxic or digestion (enzymes) inhibitory compounds. Many of these secondary plant compounds are known to be anti-metabolic or toxic towards insect pests and their presence in the cotyledons of legume seeds varies and extensive. The search for factors responsible for insect resistance yielded in the identification of antinutritional proteins such as lectin, proteinase inhibitors, amylase inhibitors and arcelin. Wild legumes are reported to produce antinutritional chemicals such as alkaloids, non-protein amino acids, and saponins in their seeds that are detrimental to the larval growth of bruchids. Various forms of resistance in pulses are mainly found to be due to the presence of genes. In a few instances, some of the compounds involved have been identified and exploited at large and their importance in legume seed defense has been discussed earlier.

Historical Background

The antinutritional protein, arcels (arc) are abundant seed storage protein that was first reported by Romero in the wild accession (PI 325690) of P. vulgaris L. that are indigenous to Middle America and South America and are potential source of protein variants used for genetic improvement of bean cultivars. Gepts, after screening 106 wild bean accessions, has reported the availability of arcels in some wild accessions. Osborn et al. have identified five putative electrophoretic variants among 22 wild Phaseolus collection from CIAT, Calif, Columbia and Western Regional Plant Introduction Station.
Excchange chromatography on CM-cellulose followed (deglycosylated) and (deglycosylated) arc4 contains polypeptides of albumin and globulin protein fractions from the quaternary structure is that of a typical legume storage protein, i.e., a multimer of similar, but not identical, sub-units. It behaves essentially as a single protein during purification.

ArcI contains polypeptides of MW 29,000 (deglycosylated) and MW 37,500 (glycosylated) 32. Arc2 contains polypeptides of MW 29,300 (deglycosylated) and 35,000 (glycosylated). Purified arc4 contains polypeptides of MW 32,000-36,000.

The purified arc5 protein fraction contains two major and one minor polypeptides (three sub-units of MW 30,000) designated arc5a, arc5b and arc5c, respectively 33. cDNA sequence for arc1 37, arc2 34, arc4 35, arc5 37, and arc6 38 has been reported earlier. Genomic sequence for arc1 37 and arc5 35 has also been reported.

Properties and Variants of Arcelin

Arcelin is controlled genetically in a simple Mendelian fashion and the expression of alleles of different arcelin variants was co-dominant with respect to each other and dominant with respect to alleles for the absence of arcelin. The genes responsible for arcelin expression are linked to the genes controlling phytohemagglutinin expression 36.

So far, seven different arcelin variants (alleles) have been identified, according to geographical locality. It is also reported that only one variant is present in any one variety 34,26,28. The molecular weight of arcelin variants is reported to be in the range of 35-42 kDa 25. Arcelins can be purified by ion-exchange chromatography on CM-cellulose followed by gel filtration on Sephacryl S-400. Examination of albumin and globulin protein fractions from the resistant accessions by SDS-PAGE shows that the major polypeptides of MW 32,000-36,000 were arcelins 36.

Native arcI is a dimeric glycoprotein of 60 kDa, built from the non-covalent association of two identical monomers. Each sub-unit contains 10 percent (w/w) neutral sugars, which belong to the high-mannose, and complex type glycans attached to three glycosylation sites. However, this is devoid of monosaccharide (simple sugars) binding properties 26-30. The native molecular weight of arcelin is estimated as MW 140,000 ± 10,000, suggesting that the arc4 molecule is a tetramer of polypeptide 39. Among various arcelins it was reported that arc2a was produced at high levels in seeds 35. Arcelin contains many homologous polypeptides, and that its quaternary structure is that of a typical legume storage protein, i.e., a multimer of similar, but not identical, sub-units. It behaves essentially as a single protein during purification.

The common bean, Phaseolus vulgaris, contains a family of plant derived insect defense proteins that comprise phytohemagglutinin (PHA), α-amylase inhibitor (α-Al) and arcelin (ARC). The genes encoded by a single locus for these proteins in P. vulgaris genome are likely to have arisen by duplication of single ancestral gene. Derived amino acid sequence obtained for PHA 41, α-amylase inhibitor 45, and arcelin 11c from cDNA clones show considerable (45-85% per cent) amino acid sequence identity and it is predicted that they have similar structures as well 45. Deglycosylated Arc1, a 29kDa protein, is reported to be structurally close to PHA and a high degree of sequence homology, both at aminoacid (58-61 per cent) and nucleotide (78 to 81 per cent) levels is reported between PHA and Arc1 34.

The main difference between arc5 and the legume lectins is the absence of the metal binding loop. Bond metals are necessary for the sugar binding capabilities of the legume lectins and stabilize the Ala-Asp cis-peptide bond. However, despite the absence of the metal binding site in arc5, the cis-peptide bond is present and the Asp residue was replaced by a Tyr residue 42.

Variations are reported to occur among the arcelin group itself. Arc4 is more closely related to arc5 than to the arc1 and arc2. Arc6 is reported to be a member of arc1 and arc2 sub-family 38. According to Sparvoli and Bollini 38, arcelins can be divided into three sub-groups: (a) arc1, arc2 and arc6, (b) arc4, and (c) arc5a and 5b. Arc3 has been classified under sub-group arc4 (Table 1).

Insecticidal Action of Arcelins

The plant defense proteins have different modes of action in protecting seeds from being infested by bruchid larvae that burrow into the seeds. The presence of arcelin in wild bean accessions was correlated with high levels of resistance to two bruchid beetle species 24,25.
The relative degrees of antibiotic of the different arcelin variants are not yet fully understood. According to John and Long\textsuperscript{34}, if different arcelins differ in their carbohydrate binding capacities, then it is likely that they may differ in their toxicity towards bruchids also. Mostly the antibiotic properties of lectins and arcelins are proposed to be due to the lysis of epithelial cells of the intestines by binding to the carbohydrate moieties of these proteins\textsuperscript{16,46}. While in other cases, \textit{viz.} the PHA binds to glycoproteins in the intestinal mucosa of insects that results in toxicity through initial binding\textsuperscript{27} and \(\alpha\)-amylase inhibitors inhibit the \(\alpha\)-amylase in the digestive tract of insects\textsuperscript{48}, the arcelins, which targets the gut, are not digestible by gut proteases of the insects\textsuperscript{51}.

Bioassay study, using arcelin, was reported to be encouraging and the survival of larvae to adults reduced by as much as 85 per cent in diets containing arc4\textsuperscript{33}. Further the antimetabolic nature of the partially purified arc4 (71 per cent) was also confirmed. In field trials also the plants containing arc1 and arc2 showed much less damage than control\textsuperscript{40}. Osborn \textit{et al.}\textsuperscript{16} have carried out bioassays with artificial seeds containing arcelins against 	extit{Zabrotes subfasciatus} and reported a marked reducing effect on larval development. Arc5 seems the most promising in conferring resistance towards insects\textsuperscript{40} followed by arc7, arc1, arc2, arc4, arc3, and arc6\textsuperscript{28,51}.

Though the toxicity of arcelin is mainly directed against insects, it is possible that arcelins may also contribute to the reduced but slightly deleterious effects of raw beans on mammals. To study the nutritional effects on the mammalian diets, Osborn \textit{et al.}\textsuperscript{16} fed the arc1 containing cooked beans to the rats and found no adverse effects on growth and metabolism. Also, Pusztai \textit{et al.}\textsuperscript{17} have reported that the antinutritional properties of arcelins could be abolished by heating the fully hydrated beans at 100°C for 10 min.

**Genetic Engineering**

Gene transfer into cultivated legume crops to impart insect resistance is a promising approach to plant protection. Transfer and expression of protease inhibitor, lectin, \(\alpha\)-amylase inhibitors etc. conferred a degree of protection against insect pest species. Osborn \textit{et al.}\textsuperscript{26} discussed the possibility of transferring arcelins through back-crossing from wild accessions to cultivar pulses, as arcelins are controlled by single Mendelian gene. Shade \textit{et al.}\textsuperscript{32} stressed upon the need to develop genetically

<table>
<thead>
<tr>
<th>Variants</th>
<th>A</th>
<th>C</th>
<th>G</th>
<th>T</th>
<th>Total bp</th>
<th>Amino acids</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arcealin (genomic clone)</td>
<td>1441</td>
<td>1014</td>
<td>682</td>
<td>1427</td>
<td>4564</td>
<td>265</td>
<td>37</td>
</tr>
<tr>
<td>(bp 3438-4169)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arcein1 (cDNA)</td>
<td>254</td>
<td>335</td>
<td>148</td>
<td>214</td>
<td>951</td>
<td>269</td>
<td>16</td>
</tr>
<tr>
<td>(bp 13-819)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arcein2 (cDNA)</td>
<td>249</td>
<td>285</td>
<td>161</td>
<td>207</td>
<td>902</td>
<td>265</td>
<td>34</td>
</tr>
<tr>
<td>(bp 1-795)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arcein4 (mRNA)</td>
<td>204</td>
<td>250</td>
<td>151</td>
<td>196</td>
<td>801</td>
<td>266</td>
<td>25</td>
</tr>
<tr>
<td>(in 801 bp mRNA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arcein5 (mRNA)</td>
<td>274</td>
<td>281</td>
<td>148</td>
<td>233</td>
<td>936</td>
<td>261</td>
<td>33</td>
</tr>
<tr>
<td>(bp 77-796)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arcein5a (genomic clone)</td>
<td>1371</td>
<td>783</td>
<td>472</td>
<td>1274</td>
<td>3900</td>
<td>261</td>
<td>35</td>
</tr>
<tr>
<td>(bp 1898-2617)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arcein5c (cDNA clone)</td>
<td>741</td>
<td>449</td>
<td>377</td>
<td>721</td>
<td>2288</td>
<td>260</td>
<td>55</td>
</tr>
<tr>
<td>(&lt;603-&gt;1385 bp)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arcein6 (mRNA)</td>
<td>298</td>
<td>301</td>
<td>90</td>
<td>236</td>
<td>1025</td>
<td>265</td>
<td>36</td>
</tr>
<tr>
<td>(bp 143-874)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
transferred plants that confer protection against insect pests of seeds in post harvest storage. Recent developments have shown that it is possible to control the storage pests through genetic engineering. The seed specific expression studies on various proteins in transgenic plants have revealed the high seed specific expression of arcelin in Phaseolus acutifolius plants. It is also reported that the arcelin-transformed plants synthesized arcelin to levels of 15 and 25 per cent of the total protein content. However, there were also plant to plant variations in arcelin expression.

Conclusions

Losses during legume seed storage are severe worldover and little viable ecofriendly technology is available to mitigate these losses. India with huge population and wide cultivable land under legumes and facing threat from different insect pests needs to invest in this direction for a productive future. Arcelins (insecticidal proteins) offer promise for a useful study in insect-plant interaction. Already farmers in Western countries and US have accepted, amidst protest from various corners, the genetically improved seeds that confer resistance to field pests, produce seeds with protection against storage pests and its benefits are manifest to them. However, one should be cautious in selecting the genes responsible for protection and these should be from the acceptable area, and comply all the prerequisites at least to confer food safety.

Acknowledgement

Financial assistance provided by the Department of Science and Technology, New Delhi to S. Janarthanan is acknowledged.

References

22. Romero J, Genetic variability in the seed protein of non-domesticated bean (Phaseolus vulgaris) L var.


Pusztai C E, Cardona C, Valor J F & Morales H. Development of lines of beans resistant to the weevil Zabrotes


55 GenBank (Accession AF 193029) NCBI, Bethesda, MD, USA, 2000.