INVOLVEMENT OF THE ARCELIN-5 PROTEIN IN THE RESISTANCE OF Phaseolus vulgaris TOWARDS Zabrotes subfasciatus

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Introduction

Arcelin, a seed storage protein found in a few wild accessions of common bean (*Phaseolus vulgaris*) is thought to be the factor responsible for the resistance of these wild beans to the insect pest *Zabrotes subfasciatus* (Mexican bean weevil), a bruchid which causes important post harvest losses in cultivated common beans from tropical countries. Six different arcelin-alleles have been identified (1-3) of which arcelin 1 and arcelin-5 seem the most promising in conferring insect resistance (4).

The arcelin-5 variant is being characterized with the aim of 1) determining the nature of arcelin 5 and 2) establishing the role of arcelin 5 in the resistance towards *Z. subfasciatus.*

Results and Discussion

Arcelin-5 proteins and cDNAs have been isolated and characterized in detail (5). The purified arcelin-5 protein fraction contains two major polypeptides and one minor polypeptide, designated arcelin 5a, arcelin 5b and arcelin 5c respectively. Arcelin 5a and arcelin 5b are glycoproteins while arcelin 5c is not glycosylated. Native arcelin 5 has a molecular mass corresponding to a dimer form and has no or very low carbohydrate-binding activity. Using amino acid sequence analysis and PCR techniques, two different arcelin-5 cDNA sequences were obtained, designated *arc5-1* and *arc5-11* (5). Both are encoding proteins of 261 amino acids with a signal peptide of 21 amino acids. The *arc5-1* and *arc5-11* cDNAs encode arcelin 5a and arcelin 5b, respectively. Sequence comparisons and protein characteristics show clearly that arcelin 5 is related to, but distinct from other arcelin variants and lectins of *P. vulgaris*.

A second part of the research focuses on the involvement of the arcelin-5 protein in the resistance towards *Z. subfasciatus*. This is done in two ways. In the first, purified arcelin-5 protein is tested for the ability to provide resistance towards *Z. subfasciatus* in artificial seeds. A first set of artificial seed tests have been done. These indicated that 1) the arcelin-5 protein seems to be less antibiotic for the Mexican bean weevil than the arcelin-1 protein and 2) the level of resistance in the arcelin-5 containing artificial seeds is significantly lower than in the G02771-seeds (i.e. the arcelin-5 containing wild line). More artificial seed tests will be conducted in which higher arcelin-5 concentrations and/or other G02771-seed protein fractions will be used. However, to determine unambiguously the involvement of arcelin-5 in the resistance it has to be proved that resistance is correlated with the genetic transfer of the arcelin-5 allele. Breeding lines containing the arcelin-5 allele indeed show high resistance levels but it cannot be excluded that resistance is caused by a factor, closely linked to the arcelin-5 allele. Therefore susceptible cultivars will be transformed with an arcelin-5 genomic clone. Seeds of transgenic plants showing high expression levels of arcelin-5 will then be tested for resistance towards *Z. subfasciatus*. For this purpose a genomic library from the G02771 accession has been constructed from which a clone, containing the arc5-1 gene, was isolated. This clone was sequenced and analyzed (6) and will be used to further assess the involvement of arcelin-5 in the insect resistance.

1. Osborn TC, *et al.* Theor. Appl. Genet. 1986;71:847-855.
2. Lioi L and Bollini R. Bean Improvement Cooperative 1989;32:28.
3. Santino A, *et al.* Plant Physiol. (Life Sci. Adv.) 1991;10:7-11.
4. Kornegay J, *et al.* Crop Sci. 1993; 33:589-594.
5. Goossens A, *et al.* Eur. J. Biochem. 1994;225:787-795.
6. Goossens A, *et al.* Plant Phisiol. (submitted) 1995.