The potential of transgenic legumes in integrated bruchid management: assessing the impact on bruchid parasitoids

Lüthi, C.*#, Álvarez-Alfageme, F., Romeis, J.
Agroscope Reckenholz-Tänikon Research Station ART, Reckenholzstrasse 191, 8046 Zurich, Switzerland.
Email: christoph.luethi@art.admin.ch

* Corresponding author
# Presenting author

Abstract

Leguminous seeds are an important staple food and source of nutrition in many countries. Bruchid beetles (Coleoptera: Bruchidae) are responsible for the greatest post-harvest losses to stored legumes. A powerful strategy to control bruchid infestations is the combination of plant resistance factors and biological control provided by parasitoids. Potent resistance factors are α-amylase inhibitors (αAI) which inhibit the starch metabolism in sensitive insects. Genetic engineering has been used to transfer αAI-1 from the common bean (*Phaseolus vulgaris*) to other leguminous plants which are subsequently protected from the attack by several bruchid species. However, there are concerns regarding the effects that the expressed insecticidal protein might have on non-target organisms. Here, we present an approach to assess the impact of αAI-1 genetically modified legumes on bruchid parasitoids.

Keywords: Risk assessment, Genetically modified plants, Non-target organisms; α-amylase inhibitor; αAI-1

1. Introduction

Legume seeds are an important source of nutrition for both humans and livestock. Their seeds are rich in proteins, carbohydrates and lipids, and they can be stored over extended periods. Additionally, the nitrogen-fixing abilities of the plants are important for the management of soil fertility. All these properties match perfectly with the requirements of small-scale, low-income farmers in developing countries.

Bruchid beetles (Coleoptera: Bruchidae) are responsible for the largest post-harvest losses to stored seeds, directly through consumption of the resource and, secondarily, through the qualitative deterioration of the commodity or the reduced stock viability. The females lay their eggs on the seed surface and the larvae burrow into the seed, where they feed and complete their development (Southgate, 1979). The beetles usually continue to multiply during seed storage, which can lead to extensive or even total losses, especially if the seeds are stored for long periods. Surface and fumigant chemical applications are thought to be the most effective methods for managing bruchid infestations. However, prohibitive costs, which limit their application to large scale or extended storage, and the risks of adverse secondary effects from such treatments, have driven the exploration of alternative strategies to manage bruchid infestations. These include biological control (Sanon et al., 1998; Gauthier et al., 1999; Schmale et al., 2006) and plant resistance factors (Ignacimuthu et al., 2000; Schmale et al., 2003; Appleby and Credland, 2004).

A crop protection tool with high potential for small-scale farmers is genetic engineering. Although the largest areas of genetically modified (GM) crop production have been in industrial countries, it's the small-scale farmers in developing countries that might benefit the most from this technology (Wambugu, 1999; Thomson, 2008). In 2008, 90% of the farmers planting GM crops were in developing countries (James, 2008). To date, all commercially cultivated insect-resistant GM crops are expressing Cry proteins derived from the soil bacterium *Bacillus thuringiensis* (Berliner) (so called *Bt* crops). The potential of insects to evolve resistance against the deployed *Bt* Cry toxins, their narrow spectrum of activity and the risk of infringing existing patents have driven the development of alternative insecticidal traits for genetic engineering, including inhibitors of digestive enzymes (Malone et al., 2008).

2. Using a Phaseolus vulgaris resistance factor for genetic engineering

The common bean, *Phaseolus vulgaris* L. (Fabaceae) and other *Phaseolus* species possess a family of evolutionary related defence proteins including phytohemagglutinin (PHA), arcelin (Arc) and α-amylase inhibitors (αAI) (Chrispeels and Raikhel, 1991). The genes of these three proteins are encoded by a single locus in the *P. vulgaris* genome (Nodari et al., 1993) and it is likely that these homologous genes have arisen by duplication of an ancestral gene. The proteins feature different modes of action and insecticidal properties against bruchids (Leavitt et al., 1977; Liener, 1986; Osborni et al., 1988; Janarthan et al., 2008; Velten et al., 2007b). αAI can strongly inhibit the activity of α-amylases. These enzymes hydrolyse starch or glycogen and play a key role in the carbohydrate metabolism of microorganisms, plants and animals. Several insects, especially those feeding on starchy seeds during any period of their life cycle like bruchid beetles, depend on α-amylases for survival (Grossi de Sa et al., 1997; Franco et al., 2002). This feature has attracted notice to αAIs, making them a promising candidate for genetic engineering. The αAI of *P. vulgaris* exists in at least two different allelic variants. The isoform found in cultivated beans is called αAI-1 (Moreno and Chrispeels, 1989); a second variant, αAI-2, is found in some wild accessions of the common bean that contain Arc as the major storage protein instead of phaseolin (Suzuki et al., 1993). Of particular interest is that the two isoforms differ in their specificity towards α-amylases. With the exception of one major storage pest, *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae), one or both αAI from the common bean have been shown to inhibit midgut amylases of major bruchid pests found worldwide (Ishimoto and Kitamura, 1992; Franco et al., 2002) (Table 1). The successful transfer of common bean αAI into tobacco plants (Altabella and Chrispeels, 1990) indicated that they could also be engineered to other plant species. Subsequently, genes encoding αAI-1 and/or αAI-2 from *P. vulgaris* were introduced by methods of genetic engineering into peas (*Pisum sativum* L.), cowpeas (*Vigna unguiculata* L.), azuki beans (*Vigna angularis* (Wildenow)) and chickpeas (*Cicer arietinum* L.) (Shade et al., 1994; Shroeder et al., 1995; Ishimoto et al., 1996; Morton et al., 2000; Solleti et al., 2008). The *α*AI gene construct introduced in these plants is regulated by flanking sequences from the seed-specific bean phytohemagglutinin PHA (*dlec*) gene. This promoter regulates the expression of the αAI restrictively to the cotyledon and embryonic axis of the developing seed (Schroeder et al., 1995), targeting exclusively seed-feeding herbivores like bruchids.

The potential of GM legumes expressing αAI to control bruchids has been confirmed in several studies. In peas, levels of expression of αAI ranged between 1.5 and 3.5% of total soluble protein, providing 100% control of *Bruchus pisorum* (L.) under glasshouse (Schroeder et al., 1995) and field conditions (Morton et al., 2000). Azuki beans expressing 0.9% αAI-1 (per dry weight) provided 100% control of the two bruchids, *Callosobruchus chinensis* L. and *Callosobruchus maculatus* F., both important pest species in East Asia (Ishimoto et al., 1996). Similarly, αAI-1-GM cowpeas and chickpeas have been reported to strongly inhibit the development of *C. chinensis* and *C. maculatus* (Sarmah et al., 2004; Solleti et al., 2008).

3. Compatibility of αAI with biocontrol agents

The potential of combining plant resistance factors together with biological control agents, especially parasitoids, has been shown to be a powerful method to control storage pests like bruchids (Schmale et al., 2003; Velten et al., 2008). One major concern regarding the use of insect-resistant GM plants is the effect that the expressed insecticidal protein might have on non-target organisms. This is especially relevant for traits with a broad spectrum of activity such as αAIs where any organism relying on α-amylases for carbohydrate digestion is potentially affected by the inhibitor. The potential impact of αAIs on bruchid parasitoids has never been evaluated. Kluh et al. (2005) extensively screened the inhibitory activity of αAI-1 against 24 insect species from eight different orders. The most sensitive species belonged to the orders of Coleoptera, Diptera and Hymenoptera. The Hymenoptera tested included an endoparasitic wasp, *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae). However, no bruchid parasitoids were included and whether they rely on α-amylases is still unknown.

4. Assessing the impact of legumes expressing αAI-1 on bruchid parasitoids

Environmental risk assessment for non-target organisms is a required step in the evaluation process of new GM plants. This is particularly true for GM crops expressing insecticidal proteins. The assessment typically follows a tiered framework which is conceptually similar to that of pesticides (Hill and Sendashonga, 2003; Romeis et al., 2008).

In an initial step one has to define what we do not want to see harmed by the GM trait. In the case of αAI-1 expressing legume seeds this is the biological control function provided by parasitic wasps that contribute to the control of bruchid species which are not or insufficiently affected by the introduced insecticidal trait. We can then develop a conceptual model describing a pathway how the presence of αAI-1 in the GM seeds can cause harm to biological control (Fig. 1). Following the different steps of the conceptual model allows a comprehensive evaluation of the risk for the parasitoids.
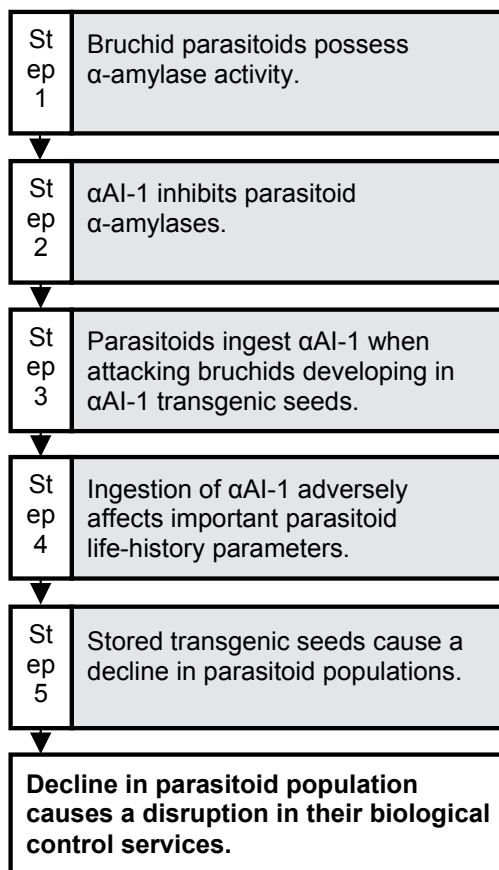
Step 1	Bruchid parasitoids possess α-amylase activity.
Step 2	αAI-1 inhibits parasitoid α-amylases.
Step 3	Parasitoids ingest αAI-1 when attacking bruchids developing in αAI-1 transgenic seeds.
Step 4	Ingestion of αAI-1 adversely affects important parasitoid life-history parameters.
Step 5	Stored transgenic seeds cause a decline in parasitoid populations.

Decline in parasitoid population causes a disruption in their biological control services.

Figure 1   Pathway on how αAI-1 from *P. vulgaris* expressed in transgenic legume seeds could harm the biological control function provided by bruchid parasitoids.

The most basic requirement to make bruchid parasitoids a potential target of αAI-1 is the presence of α-amylases. Therefore the α-amylase activity was first characterized in five different parasitic wasps which are commonly used to control bruchids (Step 1): *Anisopteromalus calandrae* (Howard) (Pteromalidae), *Dinarmus basalis* (Rondani) (Pteromalidae), *Lariophagus distinguendus* (Förster) (Pteromalidae), *Eupelmus vuilleti* (Crawford) (Eupelmidae) and *Heterospilus prosopidis* (Viereck) (Braconidae). The characterization of such enzyme activity was based on the *in vitro* characteristics of complete insect extracts. Larval and female extracts of all species were able to hydrolyze the specific substrate potato starch although a higher activity was observed in the latter. Moreover, all extracts were highly susceptible to the specific inhibitors acarbose and wheat αAI. Taken all together, these results suggest that both larvae and females rely on α-amylase activity for carbohydrate digestion. Once α-amylase activity was detected, we went to the second step. The *in vitro* susceptibility to αAI-1 was determined by using different concentrations of the inhibitor in order to construct dose-response curves for all species. The α-amylase activities from all larval and female extracts were highly susceptible to αAI-1. This finding necessitates testing the impact of αAI-1 on the parasitoids *in vivo*.

The subsequent steps of the assessment will include tritrophic experiments using different plant-host-parasitoid systems. We selected αAI-1 expressing GM cowpea and chickpea lines and three bruchid species as hosts for the parasitoids, namely *C. maculatus*, *C. chinensis* and *A. obtectus*. The two former are reported being susceptible, the latter being resistant to αAI-1 (Table 1). Bioassays with bruchids developing in GM cowpea and chickpea lines, their non-transformed isolines and other non-GM varieties will be performed to assess the variance of resistance. Measuring different life-history traits will allow verifying the susceptibility of the bruchids to αAI-1 reported in the literature. The exposure to the inhibitor at the third trophic level will also be investigated; using Western Blotting or ELISA tests, the presence of the insecticidal compound in the body of collected bruchid larvae as well as parasitoid larvae and adult females of host-feeding parasitoids will be analyzed. These data allow retracing the path of αAI-1 through the food chain and help to understand the cause of possible impacts on the non-target organisms (Step 3).

Table 1   Present known distribution of major bruchid pests and their sensitivity to αAI-1 and αAI-2 from *P. vulgaris*.

Species	Distribution Worldwide						Inhibition of midgut α-amylase	
	North America	South America	Africa	Asia	Australia	Europe	αAI-1	αAI-2
Acanthoscelides obtectus	(+)	(+)	+	+	+	+	n[3]	n[3]
Bruchus pisorum	+	+	+	(+)	+	+	y[2]	p[2]
Callosobruchus analis	+		+	(+)			y[2]	
Callosobruchus chinensis	+	+	(+)	(+)	+	+	y[1,2,3]	n[1,3]
Callosobruchus maculatus	+	+	(+)	(+)	+	+	y[1,2]	y[3]
Zabrotes subfasciatus	(+)	(+)	+	+		+	n[1,2,3]	y[1,2,3]

(+) indigenous; + established; y = yes; n = no; p = partial; [1] artificial diet; [2] transgenic seeds; [3] amylase inhibitory activity

In the fourth step, tritrophic studies will be conducted to establish the host-mediated effect of αAI-1 on the selected susceptible parasitoids. Parasitoid females will be provided GM seeds expressing αAI-1 or untransformed control seeds infested with larvae of susceptible or tolerant bruchid species and parasitoid performance (e.g., survival, fecundity) will be assessed according to Velten et al. (2007a). If detrimental effects at the third trophic level are observed, bruchid-parasitoid population dynamic experiments will be conducted in the final step to assess long-term effects on the non-target population (Wäckers, 2003).

5. Conclusions

GM legumes expressing αAI-1 from *P. vulgaris* are protected from the attack by major bruchid pests. Some species, including *A. obtectus* and *Zabrotes subfasciatus* (Boheman), however, remain unaffected by this resistant factor. It is thus important that the biological control of these species is not disrupted by the insecticidal trait expressed in the GM legumes. However, the broad range of activity of this inhibitor and its possible transfer through the food-chain necessitate a detailed analysis of the possible impacts on beneficial non-target organisms. *In vitro* inhibition studies showed that αAI-1 inhibits α-amylase activity in larvae and females of several species of bruchid parasitoids. Consequently, parasitoids might be adversely affected when developing in or feeding on bruchid pests that are not controlled by the GM trait. The assessment of these non-target effects is necessary to ensure the compatible use of biological control and the GM host plant resistance trait for a sustainable control of bruchids.

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