

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

5,600

Open access books available

138,000

International authors and editors

175M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Plant Proteinaceous α -Amylase and Proteinase Inhibitors and Their Use in Insect Pest Control

Mohammad Mehrabadi¹, Octavio L. Franco² and Ali R. Bandani¹

¹Department of Plant Protection, University of Tehran, Karaj,

²Centro de Análise Proteômicas e Bioquímicas de Brasília,
Universidade Católica de Brasília, Brasília-DF,

¹Iran

²Brazil

1. Introduction

During the evolution, plants have developed strategies to maintain favorable growth and also guarantee their survival. Enhancing the protective mechanisms, for example, is one of these strategies that allow them to successfully tolerate/resist insects, phytopathogenic microorganisms, and other unfavorable conditions (Jackson and Tailor, 1996; Malek and Dietrich, 1999; Stotz et al, 1999). Proteinaceous molecules such as α -amylase inhibitors (α -AI) and proteinase inhibitors (PIs), lectins, some hydrolyzing enzymes (e.g. b-1,3-glucanases and chitinases), and also antimicrobial peptides, are important part of protective mechanisms in plants (Fritig et al., 1998; Howe, 2008). Host plant resistance and natural plant products offer a potentially benign method for insect pest control. They are safe to the non-target beneficial organisms and human beings (Andow, 2008). This kind of plant resistance can be utilized as an economic means to reduce crop losses arising from insect pest. The wild accessions of wheat and barley do not have effective resistance and therefore plant traits that contribute to pest resistance need to be reinforced using new approaches. Thus, using the new control methods are needed such as α -amylase inhibitors, protease inhibitors, lectins and possibly δ -endotoxin (Sharma and Ortiz, 2000) to diminish reliance on insecticides.

In recent years, attentions have been focused on the idea of using digestive enzyme inhibitors that affect the growth and development of pest species (Mehrabadi et al., 2010, 2011, 2012). Inhibitors of insect α -amylase, proteinase and other plant proteins have already been demonstrated to be an important biological system in the control of insect pests (Chrispeels *et al.*, 1998; Gatehouse and Gatehouse, 1998; Morton et al., 2000; Valencia *et al.*, 2000; Carlini and Grossi-de-Sa', 2002; Svensson *et al.*, 2004; Barbosa et al., 2010). Different types of proteinaceous α -AIs are found in microorganisms, plants and animals. Cereals such as wheat, barley, rye, rice and sorghum contain small amylase inhibitors about 18 kDa in size (Abe *et al.*, 1993; Feng *et al.*, 1996; Yamagata *et al.*, 1998; Iulek *et al.*, 2000). There are different kinds of inhibitors that potentially are a good source of α -amylases inhibitors that could be used against insect pest (Franco *et al.* 2002; Svensson et al., 2004). They show

diverse structural differences thus causing different mode of actions and diverse specificity against target enzymes. Different α -amylase inhibitors have different modes of action against α -amylases for example inhibitors extracted from cereals and beans (*Phaseolus vulgaris*) have different molecular structures, leading to different modes of inhibition and different specificity against a diverse range of α -amylases. Specificity of inhibition is an important issue as the introduced inhibitor must not adversely affect the plant's own α -amylases or human amylases and must not change the nutritional value of the crop (Franco *et al.* 2002; Svensson *et al.*, 2004).

Proteinase inhibitors are capable of interfering with insect protein digestion by binding to digestive proteases of phytophagous insects, resulting in an amino acid deficiency thus affecting insect growth and development, fecundity, and survival (Lawrence and Koundal 2002; Oppert *et al.* 2003; Azzouz *et al.* 2005). They along with α -amylases inhibitors constitute major tools for improving the resistance of plants to insects. Transgenic plants expressing serine and cysteine proteinase inhibitors have shown resistance to some insect pest species including Lepidoptera and Coleoptera (De Leo *et al.* 2001; Falco and Silva-Filho 2003; Alfonso-Rubi *et al.* 2003). PIs are the products of single genes, therefore they have practical advantages over genes encoding for complex pathways and they are effective against a wide range of insect pests, i.e. transferring trypsin inhibitor gene from *Vigna unguiculata* to tobacco conferred resistance against lepidopteran insect species such as *Heliothis* and *Spodoptera*, and coleopteran species such as *Diabrotica* and *Anthonomus* (Hilder *et al.* 1987).

2. Insect α -amylases

α -Amylases (α -1,4-glucan-4-glucanohydrolases; EC 3.2.1.1) are one of the most widely enzyme complexes encountered in animals, higher and lower plants, and microbes. Because of their importance in organism growth and development, these enzymes from different origins including bacteria, nematodes, mammals and insects have been purified and their physical and chemical properties characterized (Baker, 1991; Nagaraju and Abraham, 1995; Zoltowska, 2001; Rao *et al.*, 2002; Valencia *et al.*, 2000; Mendiola-Olaya *et al.*, 2000; Oliveira-Nato *et al.*, 2003 Mohammed, 2004; Bandani *et al.*, 2009; Mehrabadi *et al.*, 2009). Many phytophagous insects, like stored product insects, live on a polysaccharide-rich diet and are dependent on their α -amylases for survival (Mendola-Olaya *et al.* 2000; Boyd *et al.* 2002; Mehrabadi *et al.*, 2011). They convert starch to maltose, which is then hydrolyzed to glucose by α -glucosidase. In insects, only α -amylases that hydrolyse α -1,4- glucan chains such as starch or glycogen have been found (Terra *et al.* 1999).

According to Terra and Ferreira (1994), insect α -amylases generally have molecular weights in the range 48–60 kDa, pI values of 3.5–4.0, and K_m values with soluble starch around 0.1%. pH optima generally correspond to the pH prevailing in midguts from which the amylases were isolated. Insect amylases are calcium-dependent enzymes, and are activated by chloride with displacement of the pH optimum. Activation also occurs with anions other than chloride, such as bromide and nitrate, and it seems to depend upon the ionic size (Terra and Ferreira, 1994).

Although the sequences of several insect α -amylases are known, the best characterized insect α -amylase whose 3D-structure has been resolved is *Tenebrio molitor* (TMA) α -

amylase. The three-dimensional model of TMA consists of a single polypeptide chain of 471 amino acid residues, one calcium ion, one chloride ion and 261 water molecules. The enzyme consists of three distinct domains, A (residues 1 to 97 and 160 to 379), B (residues 98 to 159) and C (residues 380 to 471) (Strobl et al, 1998). A representation of the overall polypeptide folds, as well as the location of the bound ions and the residues presumably involved in catalysis. Domain A is the central domain with $(\beta / \alpha)_8$ -barrels comprises the core of the molecule and also includes the catalytic residues (Asp 185, Glu 222 and Asp 287). Two other domains (Domains B and C) are opposite each other, on each side of domain A. The Ca^{2+} binding site in TMA is located at the interface of the domain A central β -barrel and domain B. This ion is important for the structural integrity of TMA. TMA has also chloride-binding site on the same side of the β -barrel as the catalytic and the calcium-binding site, in the vicinity of both. Insect α -amylases are closely related to mammalian α -amylases (Strobl et al., 1997). The most striking difference between mammalian and insect α -amylases is the presence of additional loops in the vicinity of the active site of the mammalian enzymes (Strobl et al., 1998a). α -Amylases are the most important digestive enzymes of many insects that feed exclusively on seed products during larval and/or adult life. When the action of the amylases is inhibited, nutrition of the organism is impaired causing shortness in energy.

3. Proteinaceous α -amylase inhibitors from plants

α -AIs are abundant in microorganisms, higher plants, and animals (Da Silva et al., 2000; Toledo et al., 2007). These organisms produce a large number of different protein inhibitors of α -amylases in order to regulate the activity of these enzymes. α -Amylase inhibitors can be extracted from several plant species including legumes (Marshall and Lauda, 1975; Ishimoto et al., 1996; Grossi-de-Sa et al., 1997) and cereals (Abe et al., 1993; Feng et al., 1996; Yamagata et al., 1998; Franco et al., 2000; Iulek et al., 2000). Diverse α -amylase inhibitors reveal different characteristics against various α -amylases (Franco et al., 2002). In Table 1 inhibitory activity of α AIs from different sources are reviewed. α -AIs are naturally used by plants as a defense mechanism against insect pests (Ishimoto et al., 1989; Kluh et al., 2005). Moreover, there is a great interest to use α -AIs for control of insect pest and also to use them for production of transgenic plants that are resistant against insect pest (Gatehouse et al., 1998; Chrispeels et al., 1998; Gatehouse and Gatehouse, 1998; Valencia et al., 2000; Yamada et al., 2001). Inhibitors to insect α -amylase have already been demonstrated to be an important biological system in the control of insect pests (Franco et al., 2002; Carlini and Grossi-de-Sa', 2002; Svensson et al., 2004). The expression of α -amylase inhibitors has been showed to be effective in transgenic plants. The expression of the cDNA encoding α AI-I into some plants such as pea (*Pisum sativum* L.) and azuki bean (*Vigna angularis* L.) against bruchid beetle pests (Coleoptera: Bruchidae) has been well documented for showing ability of these inhibitors to be used as plant resistance factors against some species of insect pests (Ishimoto et al., 1989; Yamada et al., 2001; Kluh et al., 2005). Pea and azuki transgenic plants expressing α -amylase inhibitors from common beans (α -AI) were completely resistant to the *Bruchus pisorum* and *Callosobruchus chinensis* weevils (Morton et al. 2000). Rye α -amylase inhibitor expressed in transgenic tobacco seeds (*Nicotiana tabacum*) caused 74% mortality in *Anthonomus grandis* first instar larvae when transgenic seed flour mixture used in artificial diet (Dias et al., 2010).

α-Amylase inhibitor	Plant origin	Target pest	Test condition	Reference
α BIII	<i>Secale cereale</i>	<i>Anthonomus grandis</i> <i>Acanthoscelides obtectus</i> , <i>Zabrotess subfasciatus</i> and	transgenic tobacco In vitro	(Dias et al., 2005, 2010)
Ric c 1 and Ric c 3	<i>Ricinus communis</i>	<i>Callosobruchus maculatus</i> , <i>Zabrotes subfasciatus</i> , <i>Tenebrio molitor</i>	Feeding assay	(Do Nascimento et al., 2011)
Baru seed extract	<i>Dipteryx alata</i>	<i>Callosobruchus maculatus</i>	In vivo	(Bonavides et al., 2007)
α -AI-1 and α -AI2	<i>Phaseolus coccineus</i>	<i>Hypothenemus hampei</i> <i>Tecia solanivora</i>	In vitro	(Valencia-Jiménez et al., 2008)
α AI-Pc1	<i>Phaseolus coccineus</i>	<i>Hypothenemus hampei</i>	Transgenic plant	(de Azevedo et al., 2006)
α -AI1, α -AI2	<i>Phaseolus vulgaris</i>	coffee berry borer pest <i>Callosobruchus maculatus</i> <i>Callosobruchus chinensis</i> <i>Zabrotes subfasciatus</i> <i>Sitophilus oryzae</i> <i>Acanthoscelides obtectus</i> <i>Cryptolestes ferrugineus</i> <i>Cryptolestes pusillus</i> <i>Oryzaephilus surinamensis</i> <i>Sitophilus granarius</i> <i>Tribolium castaneum</i> <i>T. castaneum</i> <i>Drosophila melanogaster</i> <i>Sarcophaga bullata</i> <i>Aedes aegypti</i> <i>Monomorium pharaonis</i> <i>Apis mellifica</i> <i>Venturia canescens</i> <i>Ephestia cautella</i> <i>E. elutella</i> <i>E. kuehniella</i> <i>Manduca sexta</i> <i>Ostrinia nubilalis</i> <i>Blattella germanica</i> <i>Liposcelis decolor</i> <i>Acheta domesticus</i> <i>Eurydema oleracea</i> <i>Graphosoma lineatum</i>	Transgenic plant In vivo	(Barbosa et al., 2010; Solleti et al., 2008; Nishizawa et al., 2007; Ignacimuthu, and Prakash, 2006; Kluh et al., 2005)
VuD1	<i>Vigna unguiculata</i>	<i>Acanthoscelides obtectus</i> and <i>Zabrotes subfasciatus</i>	In vitro	(Pelegriani et al., 2008)
VrD1	<i>Vigna radiata</i>	<i>Tenebrio molitor</i>	In silico	(Liu et al., 2006)
KPSI	<i>Vigna radiata</i>	<i>Callosobruchus Maculatus</i>	In vivo	(Wisessing, 2010)

α -Amylase inhibitor	Plant origin	Target pest	Test condition	Reference
DR1-DR4	<i>Delonix regia</i>	<i>Callosobruchus maculatus</i> <i>Anthonomus grandis</i>	In vivo	(Alves, 2009)
(AI)-1 and (AI)-1	<i>Pisum sativum</i>	<i>Bruchus pisorum</i>	Transgenic plant	(De Sousa-Majer, et al., 2007)
CpAI	<i>Carica papaya</i>	<i>Callosobruchus maculatus</i>	In vivo	(Farias, et al., 2007)
α -AIs from <i>Triticum aestivum</i>	<i>Triticum aestivum</i>	<i>Eurygaster integriceps</i> <i>Tenebrio molitor</i> <i>Rhyzopcrtha dominica</i> <i>Callosobruchus maculatus</i>	In vitro In vivo	(Mehrabadi et al., 2010, 2012; Zoccatelli et al., 2007; Cinco-Moroyoqui et al., 2006; Amirhusin, et al., 2004)
0.19 AI 0.53 AI	<i>Triticum aestivum</i>	<i>Acanthoscelides obtectus</i>	In vivo	(Franco et al., 2005)
BIII	<i>Secale cereale</i>	<i>Acanthoscelides obtectus</i> , <i>Zabrotess subfasciatus</i> <i>Anthonomus grandis</i>	In vivo In vitro	(Dias, et al., 2005; Oliveira-Neto et al., 2003)
SPAI1-SPAI4	<i>Ipomoea batatas</i>	<i>Araecerus fasciculatus</i> <i>Sitophilus oryzae</i> <i>Cylas formicarius elegantulus</i> <i>Tribolium castaneum</i>	In vitro	(Rekha, et al., 2004)
TAI1,TAI2 C154, C178, C249, C439, C487	<i>Colocasia esculenta</i>	<i>Araecerus fasciculatus</i> <i>Sitophilus oryzae</i> <i>Cylas formicarius elegantulus</i> <i>Tribolium castaneum</i>	In vitro	(Rekha, et al., 2004)
α -PPAI and α -ZSAI	<i>Ficus</i> sp.	<i>Callosobruchus maculatus</i> <i>Zabrotess subfasciatus</i>	In vitro	(Bezerra et al., 2004)
PpAI	<i>Pterodon pubescens</i>	<i>Callosobruchus maculatus</i>	In vivo	(Silva et al., 2007)

Table 1. Plant α -amylase inhibitors and their activities against insect pests (literature review since 2002).

3.1 Plant α -amylase inhibitor classes

Based on structural similarity, there are six different proteinaceous α -amylase inhibitors with plant origin including lectin-like, knottin-like, CM-proteins, Kunitz-like, c-purothionin-like, and thaumatin-like (Richardson, 1990) (Table 2).

Inhibitor class	Plant origin	Target	Residues number (aa)	Names	References
Legum lectin-like	Common bean	Insect, Mammalian, Fungal	240-250	α AI1, α AI2	(Marshall and Lauda, 1975; Ho and Whitaker, 1993)
Knottin-like	Amaranth	Insect	32	AAI	(Chagolla-Lopez et al., 1994)
Kunitz-like	Wheat, Barley, Rice, maize, cowpea	Insect, Plant	176-181	BASI, RASI, WASI	(Mundy et al., 1983; 1984; Swensson et al., 1986; Ohtsubo and Richardson, 1992; Iulek et al., 2000; Alves et al., 2009)
γ - Purothionin	Sorghum	Insect, Mammalian	47-48	SI α 1, SI α 2, SI α 3	(Bloch Jr and Richardson, 1994)
Thaumatin-like	Maize	Insect	173-235	Zeamatin	(Schmioler-O'Rourke and Richardson, 2001; Franco et al., 2002)
CM-proteins	Wheat, Barley, Rey, ragi	Insect, Mammalian, Bacteria	124-160	0.19,0.28,0.53, RATI (RBI), RP25, WRP26, BMAI-1	(Campos and Richardson, 1983; Mundy et al., 1984; Franco et al., 2000, 2002; Swensson et al., 2004)

Table 2. Classification of plant α -amylase inhibitors based on structural similarity (Richardson, 1990).

3.1.1 Lectin-like inhibitors

There have been particular attentions on lectin-like inhibitors and they are toxic against several insect pests (Ishimoto and Kitamura, 1989; Huesing et al., 1991a; Ishimoto and Chrispeels, 1996; Grossi-de-Sa et al., 1997, Kluh et al., 2005; Karbache et al., 2011). α AI-1 and α AI-2, Two lectin-like inhibitors, were identified in common white, red and black kidney beans (Ishimoto and Chrispeels, 1996;). They show different specificity against α -amylases because of the mutation in their primary structure (Grossi de Sa et al., 1997). α AI-1 inhibits mammalian α -amylases and several insect amylases, but it is not active against Mexican bean weevil (*Zabrotes subfasciatus*). On the other hand, α AI-2 does not inhibit the α -amylases recognized by α AI-1 but inhibits the α -amylase of *Z. subfasciatus* (Ishimoto and Chrispeels, 1996; Kluh et al., 2005).

3.1.2 Knottin-like inhibitors

The major α -amylase inhibitor (AAI) present in the seeds of *Amaranthus hypocondriacus*, is a 32-residue-long polypeptide with three disulfide bridges (Chagolla-Lopez et al., 1996). AAI strongly inhibits α -amylase activity of *Tribolium castaneum* and *Prostephanus truncates*, however, does not inhibit proteases and mammalian α -amylases. AAI is the smallest proteinaceous inhibitor of α -amylases yet described. Its residue conservation patterns and

disulfide connectivity are related to the squash family of proteinase inhibitors, to the cellulose binding domain of cellobiohydrolase, and to omega-conotoxin, i.e. knottins. The three-dimensional model of AAI contains three antiparallel β strands and it is extremely rich in disulfides (Carugo et al., 2001).

3.1.3 Kunitz-type

Kunitz-like α -amylase inhibitors commonly found in cereals such as barley, wheat and rice (Micheelsen et al., 2008; Nielsen et al., 2004). Recently, they have also reported from legums, e.g. Cowpea (*Vigna unguiculata*) (Alves et al., 2009). Kunitz-like α -amylase inhibitors from Cowpea were active against both insect and mammals α -amylase with different intensity (Alves et al., 2009). α -Amylase/subtilisin inhibitors (BASI) are the most studied inhibitors of the Kunitz-like trypsin inhibitor family (Melo et al., 2002), that have bifunctional action i.e. as a plant defense and also as a regulator of endogenous α -amylase action (Micheelsen et al., 2008; Nielsen et al., 2004). The structure of BASI consists of two disulfide bonds and a 12-stranded β -barrel structure which belongs to the β -trefoil fold family. The interaction of Kunitz-like α -amylase inhibitors with the barley α -amylase 2 (AMY2) revealed a new kind of binding mechanisms of proteinaceous α -amylase inhibitors since calcium ions modulate the interaction (Melo et al., 2002).

3.1.4 γ - Purothionin type

The members of this family contain inhibitors with 47 – 48 amino acid residues that show strongly inhibition activity against insect α -amylases (Bloch Jr and Richardson, 1991). SI α -1, SI α -2 and SI α -3 are three isoforms isolated from *Sorghum bicolor* and showed inhibitory activity against digestive α -amylases of cockroach and locust, poorly inhibited *A. oryzae* α -amylases and human saliva. These inhibitors did not show inhibitory activity on the α -amylases from porcine pancreas, barley and *Bacillus* sp. (Bloch Jr and Richardson, 1991). The three isoforms contain eight cysteine residues forming four disulfide bonds (Nitti et al., 1995).

3.1.5 CM- proteins

CM (chloroform-methanol)-proteins are a large protein family from cereal seeds containing 120 –160 amino acid residues and five disulfide bonds (Campos and Richardson, 1983; Halford et al., 1988). Cereal-type is also refers to these inhibitors since they are present in cereals. CM-proteins show a typical double-headed α -amylase/trypsin domain (Campos and Richardson, 1983). This feature make it possible that they show inhibitory activity against α -amylases (Barber et al., 1986a) and trypsin-like enzymes (Barber et al., 1986b; De Leo et al., 2002) separately or show α -amylases/ trypsin-like inhibitory activity at the same time (Garcia- Maroto et al., 1991). The CM protein family includes lipid transfer proteins (Lerche and Poulsen, 1998; Svensson et al., 1986) and proteins related to cold tolerance (Hinch, 2002). The α -amylase inhibitor 0.19, one of the most studied inhibitor of this family, has a broad specificity and inhibits α -amylases from insects, birds and mammal (Titarenko et al., 2000; Franco et al., 2000; Franco et al., 2002; Oneda et al., 2004). It has 124 amino-acid residues and acting as a homodimer (Oda et al., 1997; Franco et al., 2000). The X-ray crystallographic analysis of 0.19 AI demonstrated that each subunit is composed of four major α -helices, one one-turn helix, and two short antiparallel β -strands. The subunits in a

dimer are related each other by non-crystallographic 2-fold axis, and the interface is mainly composed of hydrophobic residues (Oda et al., 1997).

3.1.6 Thaumatin-like

This family contains proteins with molecular weight about 22 kDa, which are homologous with the intensely sweet protein thaumatin from fruits of *Thaumatococcus daniellii* Benth, thus they are called thaumatin-like (Cornelissen et al., 1986; Vigers., 1991; Hejgaard et al., 1991). Although thaumatin-like proteins is a homologue of the sweet protein thaumatin and exhibit α -amylase inhibitory activity, however, thaumatin and other related proteins do not show inhibitory activity against α -amylases (Franco et al., 2002; Svensson et al., 2004 and references therein). Zeamatin from maize is the best-characterized member of this family which inhibits insect but not mammalian α -amylases. Zeamatin has 13 β strands, 11 of which form a β sandwich at the core of protein (Batalia et al., 1996). Zeamatin has been applied as antifungal drugs because it binds to β -1,3-glucan and permeabilizes fungal cells resulting in cell death (Roberts and Selitrennikoff, 1990; Franco et al., 2000).

4. Insect digestive proteinases

Proteinases, which are also known as endopeptidases, enroll an important function in protein digestion. These enzymes begin the protein digestion process by breaking internal bonds in proteins. The amino acid residues vary along the peptide chain, therefore, different kind of proteinases are necessary to hydrolyze them. Based on active site group and their correspond mechanism, digestive proteinases can be classified as serine, cysteine, and aspartic proteinases (Terra and Ferreira, 2012). Serine, cysteine are the most widespread proteinases in insect digestive system.

Serine proteinases (EC 3.4.21) have the active site composed of serine, histidine, and aspartic acid residues (also called catalytic triad). Trypsin (EC 3.4.21.4), chymotrypsin (EC 3.4.21.1), and elastase (EC 3.4.21.36) are the major digestive enzymes of this family that usually work at alkalin pH. These enzymes differ in structural features that are associated with their different substrate specificities. Trypsin are endoproteases that attack proteins at residues of arginine and lysine. Generally, insect trypsins have molecular masses in the range 20–35 kDa, pI values 4–5, and pH optima 8–10 (Terra and Ferreira, 2012). Trypsin occurs in the majority of insects, with the remarkable exception of some hemipteran species and some taxa belonging to the series Cucujiformia of Coleoptera like Curculionidae (Terra and Ferreira, 1994). Nevertheless, some heteropteran Hemiptera have trypsin in the salivary glands (Zeng et al., 2002). Chymotrypsin enzymes attack proteins at aromatic residues (e.g., tryptophan). Insect chymotrypsins usually have molecular masses of 20–30 kDa and pH optima of 8–11 (Terra and Ferreira, 1994). Similar to trypsin, chymotrypsin is also distributed in the majority of insects (Terra and Ferreira, 1994), including those purified from Lepidoptera (Peterson et al., 1995; Volpicella et al., 2006), Diptera (de Almeida et al., 2003; Ramalho-Ortigão et al., 2003), Hemiptera (Colebatch et al., 2002), Hymenoptera (Whitworth et al., 1998), Siphonaptera (Gaines et al., 1999) and Coleoptera (Oliveira-Neto et al., 2004; Elpidina et al., 2005).

Cysteine proteinases occur in the digestive system of insects (Rawlings and Barrett, 1993). These enzymes are also found in other tissue of insects, indicating that they are associated

with other functions in insect (Matsumoto et al., 1997). Cysteine proteinases have their optimum activity in the alkaline range (Bode and Huber, 1992; Oliveira et al., 2003). It has been revealed that cathepsin L-like enzymes are the only quantitatively important member of cysteine proteinases presented in midgut of insects. Digestive cathepsin L-like enzymes have been purified from *Diabrotica virgifera* (Coleoptera: Cucujiformia) (Koiwa et al., 2000), *Acyrtosiphon pisum* (Hemiptera: Sternorrhyncha) (Cristofolletti et al., 2003), *T. molitor* (Coleoptera: Cucujiformia) (Cristofolletti et al., 2005), *Sphenophorus levis* (Coleoptera: Curculionidae) (Soares-Costa et al., 2011; Fonseca et al., 2012), and *Triatoma brasiliensis* (Reduviidae, Triatominae) (Waniek et al., 2012).

5. Proteinase inhibitors from plants

PIs are a natural plant defensive mechanism against insect herbivores which were viewed as promising compounds for developing insect resistance transgenic crops that over-express PIs (Gatehouse, 2011). PIs have found in animals, plants (particularly legumes and cereals), and microorganisms. Most storage organs such as seeds and tubers contain 1-10% of their total proteins as PIs with different biochemical and structural properties inhibiting different types of proteases (Volpicella et al., 2011). PIs play an important role in different physiological functions of plants including as storage proteins, and regulators of endogenous proteolytic activity (Ryan, 1990), modulators of apoptotic processes or programmed cell death (Solomon et al., 1999), and defense components associated with the resistance of plants against insects and pathogens (Lu et al., 1998; Pernas et al., 1999). Green and Ryan (1972) pioneer works revealed the roles of PIs in the plant-insect interaction. They showed induction of plant PIs in response to attack of insects and pathogen and named this induction as “defense-response” of the plant against the pests. Production of PIs that inhibit digestive herbivore gut proteases inspired the field of plant- insect interactions and became an outlandish example of induced plant defenses. Since then, several PIs of insect proteinases have been identified and characterized (Garcia-Olmedo et al. 1987; Lawrence and Koundal 2002). Despite insects that feed on sap or seeds, most phytophagous insects are nutritionally limited by protein digestion. Since plant tissues are nitrogen deficient compared to insect composition, and the main source of nitrogen available to the insect is protein (Gatehouse, 2011). Therefore, their proteinases have an important role in digestion of proteins and maintaining of needed nitrogen. Inactivation of digestive enzymes by PIs results in blocking of gut proteinases that leads to poor nutrient utilization, retarded development, and death because of starvation (Jongsma and Bolter 1997; Gatehouse and Gatehouse, 1999).

There have been considerable number of reviews on plant PIs describing their classification (Turra et al., 2011; Volpicella et al., 2011), biochemical and structural properties (Antao and Malcata, 2005; Bateman and James, 2011; Oliva et al., 2011), their role in plant physiology (Schaller, 2004; Salas et al., 2008; Roberts and Hejgaard, 2008), insect-plant co-evolution (Jongsma and Beekwilder, 2011), and their application in different areas including pest control (Lawrence and Koundal, 2002; Gatehouse, 2011), nutritional (Clemente et al., 2011) as well as pharmaceutical (Gomes et al., 2011) applications.

5.1 Plant proteinase inhibitors classes

PIs are classified based on the type of enzyme they inhibit: Serine protease inhibitors, cysteine protease inhibitors, aspartic protease inhibitors, or metalloprotease

inhibitors (Ryan, 1990; Mosolov, 1998; Bode and Huber, 2000). Plant serine proteinase inhibitors further sub-classified to a number of subfamilies based on their amino acid sequences and structural properties known as Kunitz type, Bowman-Birk type, Potato I type, and Potato II type inhibitors (Bode and Huber, 1992). The families of PIs could not, however, be grouped on the basis of the catalytic type of enzymes inhibited, since a number of families contain cross-class inhibitors. Despite cysteine and metalcarboxy inhibitor families, all other reported families of PIs contain inhibitors of serin proteases. (Volpicella et al., 2002). The proteins in Kunitz-like family, for instance, generally inhibit serine proteinases, besides they also include inhibitors of cysteine and aspartate proteases (Heibges et al., 2003). There are some exceptions, however, that PIs families may have not inhibitors of serine proteases such as aspartic protease inhibitors in Kunitz and cystein families and also potato cystein protease inhibitors that belongs to Kunitz family (Volpicella et al., 2002).

6. Transgenic plants expressing digestive enzyme inhibitors

It seems obvious that the prospective amylase and proteinase inhibitors can function as a biotechnological tool for the discovery of novel bioinsecticides or in the construction of transgenic plants with enhanced resistance toward pests and pathogens.

Since Johnson et al. (1989) expressed proteinase inhibitors in transgenic tobacco providing enhanced resistance against *Manduca sexta* larvae, hundreds of reports have been produced in this specific issue. As previously described, proteinase inhibitors could act on the digestive enzymes of insect herbivores reducing food digestibility. Attempts to achieve this defense mechanism in plants, genetic engineering have used over-expression of both exogenous and endogenous proteinase inhibitors (Gatehouse, 2011).

Among several targets, Lepidopteran has been clearly focused, since they are important groups of crop insect-pests in the world. Until now the only commercially accessible transgenes for control of these insect pests encode Cry *Bacillus thuringiensis* (Bt) toxins and the Vip3Aa20 toxin (United States Environmental Protection Agency, 2009). Several trials have been performed by using proteinase inhibitors. For example the mustard trypsin inhibitor (MTI-2) was expressed at different levels in transgenic tobacco, Arabidopsis and oilseed rape lines. The three plants were challenged against different lepidopteran insect-pests, including *Plutella xylostella* (L.), which was extremely sensible to MTI-2 ingestion being completely exterminated (de Leo et al., 2001). Furthermore MTI-2 was also expressed at different levels in transgenic tobacco lines and was further appraised by feeding of the lepidopteran larvae, *Spodoptera littoralis* (de Leo and Galerani et al., 2002). A surprising result was obtained. *S. littoralis* larvae feed on transgenic tobacco expressing MTI-2 were unaffected. However, significant reduction on fertility was obtained suggesting that multiple effects could be obtained with a single proteinase inhibitor. In this view, several research groups have produced and evaluated transgenic plants synthesizing proteinase inhibitors and attacked by Lepidoptera pests. Among inhibitors expressed in transgenic plants were NaPI, the *Nicotiana alata* proteinase inhibitor and also the multidomain potato type II inhibitor that is produced at enhanced levels in the female reproductive organs of *N. alata* (Dunse et al., 2010). The individual inhibitory domains of NaPI target trypsin and chymotrypsin, from digestive tract of lepidopteran larval pests. While feeding on NaPI, dramatically reduced the *Helicoverpa punctigera* growth, surviving larvae exhibited high

levels of chymotrypsin resistant to inhibition by NaPI. In order to solve this problem, NaPI was expressed in synergism with *Solanum tuberosum* potato type I inhibitor (StPin1A), which strongly inhibited NaPI-resistant chymotrypsins. The mutual inhibitory effect of NaPI and StPin1A on *H. armigera* larval growth was observed both in laboratory conditions as well as in field trials of transgenic plants.

Improved crop protection achieved using mixtures of inhibitors in which one class of proteinase inhibitor is utilized to contest the genetic ability of an insect to adapt to a additional class of proteinase inhibitor. Furthermore, amylase inhibitors have also been utilized as defense factors against insects in genetic modified plants. Several amylase inhibitors have been expressed in different plants. However the expression of α -amylase inhibitors (α -AI) from scarlet runner bean (*Phaseolus coccineus*) and common bean (*Phaseolus vulgaris*) has been extremely protective in genetic modified plants, showing enhanced shelter against pea weevils (Shade et al., 1994; Schroeder et al., 1995), adzuki bean (Ishimoto et al., 1996), chickpea (Sarmah et al., 2004; Ignacimuthu et al., 2006, Campbell et al., 2011) and cowpea (Solleti et al., 2008). Furthermore, transgenic pea showed enhanced defense against the pea weevil *Bruchus pisorum* was shown under field conditions (Morton et al., 2000). All these trials associated the α -AI expression with the seed-specific promoter of phytohemagglutinin from *P. vulgaris*.

Moreover other crops, in addition to legumes, were also transformed with amylase inhibitors. The Rubiaceae *Coffea arabica* was also engineered with α -AI1 under control of phytohemagglutinin promoter (Barbosa et al., 2010). The presence of this gene was observed by PCR and Southern blotting in six regenerated transgenic T1 coffee plants. Immunoblotting and ELISA experiments using antibodies against α -AI1 revealed the presence of this inhibitor at a concentration of 0.29 % in seed extracts. The presence of this inhibitor was able to cause a clear inhibitory activity on digestive enzymes of *Hypotenemus hampei* suggesting a possible protective effect.

Also, an α -amylase inhibitor from cereal-family (BIII) from rye (*Secale cereale*) seeds was also cloned and expressed initially in *E. coli* showing clear activity toward α -amylases of larvae of the coleopteran pests *Acanthoscelides obtectus*, *Zabrotess subfasciatus* and *Anthonomus grandis* (Dias et al. 2005). BIII inhibitor was also expressed under control of phytohemagglutinin promoter in tobacco plants (*Nicotiana tabacum*). Besides, the occurrence of BIII-rye gene and further protein expression were confirmed. Immunological analyzes indicated that the recombinant inhibitor was produced in concentration ranging from 0.1% to 0.28% (w: w). Bioassays using transgenic seed flour for artificial diet caused 74% mortality for cotton boll weevil *A. grandis* suggesting that rye inhibitor could be an auspicious biotechnological tool for yield transgenic cotton plants with an improved resistance to weevil (Dias et al., 2010).

7. Summary

While important protection against insect pests has been routinely achieved, the transgenic plants do not show levels of resistance considered commercially possible. As a consequence of selective pressures, insect herbivores have developed various adaptation mechanisms to overcome the defensive effects of plant inhibitors. Common polyphagous crop pests are well adapted to avoid a wide range of different inhibitors, which have only limited effects.

Multiple strategies have been attempted to improve effectiveness of digestive enzyme inhibitors towards insects, including selection for inhibitory activity toward digestive enzymes, mutagenesis for novel inhibitory activity, and engineering multifunctional inhibitors. However, digestive enzyme inhibitors have only been used in genetic modified crops in mishmash with other insecticidal genes. In genetically engineered cotton plants which express Bt toxins, the CpTI gene has been employed as an additional transgene to improve protection against lepidopteran larvae. This gene combination indicates the only commercial disposition of a proteinase inhibitor transgene to date, with Bt/CpTI cotton grown on over 0.5 million hectares in 2005. Until now, no amylase inhibitor was commercially utilized. Future predictions for using digestive enzyme inhibitor genes to boost insect resistance in transgenic crops will require reconsideration of their mechanisms of action, particularly in disturbing processes other than ingestion.

8. Acknowledgement

The authors would like to acknowledge Iran National Science Foundation (INSF) for their financial support. This work was funded by a grant (No. 86025.11) from INSF.

9. References

- Alves, D. T., Vasconcelos, I. M., Oliveira, J. T. A., Farias, L. R., Dias, S. C., Chiarello, M. D., et al. (2009). Identification of four novel members of kunitz-like α -amylase inhibitors family from *Delonix regia* with activity toward coleopteran insects. *Pesticide Biochemistry and Physiology*, 95(3), 166-172.
- Amirhusin, B., Shade, R. E., Koiwa, H., Hasegawa, P. M., Bressan, R. A., Murdock, L. L., et al. (2004). Soyacystatin N inhibits proteolysis of wheat α -amylase inhibitor and potentiates toxicity against cowpea weevil. *Journal of Economic Entomology*, 97(6), 2095-2100.
- Antao, C.M.; Malcata, F.X. (2005). Plant serine proteases: biochemical, physiological and molecular features. *Plant Physiology and Biochemistry*, 43(7), 637-650.
- Barbosa AE, Albuquerque EV, Silva MC, Souza DS, Oliveira-Neto OB, Valencia A, Rocha TL, Grossi-de-Sa MF. (2010). Alpha-amylase inhibitor-1 gene from *Phaseolus vulgaris* expressed in *Coffea arabica* plants inhibits alpha-amylases from the coffee berry borer pest. *BMC Biotechnology*, 17, 10:44.
- Bateman, K. S., & James, M. N. (2011). Plant protein proteinase inhibitors: Structure and mechanism of inhibition. *Current Protein & Peptide Science*, 12(5), 340-347.
- Bezerra, I. W. L., Teixeira, F. M., Oliveira, A. S., Araújo, C. L., Leite, E. L., Queiroz, K. C. S., et al. (2004). α -Amylase inhibitors from ficus sp. seeds and their activities towards coleoptera insect pests. *Protein and Peptide Letters*, 11(2), 181-187.
- Bloch Jr., C., & Richardson, M. (1991). A new family of small (5 kDa) protein inhibitors of insect α -amylases from seeds of Sorghum (*Sorghum bicolor* (L) moench) have sequence homologies with wheat γ -purothionins. *FEBS Letters*, 279(1), 101-104.
- Bonavides, K. B., Pelegrini, P. B., Laumann, R. A., Grossi-De-Sá, M. F., Bloch Jr., C., Melo, J. A. T., et al. (2007). Molecular identification of four different α -amylase inhibitors from Baru (*Dipteryx alata*) seeds with activity toward insect enzymes. *Journal of Biochemistry and Molecular Biology*, 40(4), 494-500.

- Campbell PM, Reiner D, Moore AE, Lee RY, Epstein MM, Higgins TJ. (2011). Comparison of the α -amylase inhibitor-1 from common bean (*Phaseolus vulgaris*) varieties and transgenic expression in other legumes--post-translational modifications and immunogenicity. *Journal of Agricultural and Food Chemistry*, 59 (11), 6047-6054.
- Campos, F. A. P., & Richardson, M. (1983). The complete amino acid sequence of the bifunctional α -amylase/trypsin inhibitor from seeds of ragi (indian finger millet, *Eleusine coracana* Gaertn.). *FEBS Letters*, 152(2), 300-304.
- Chagolla-Lopez, A., Blanco-Labra, A., Patthy, A., Sánchez, R., & Pongor, S. (1994). A novel α -amylase inhibitor from Amaranth (*Amaranthus hypocondriacus*) seeds. *Journal of Biological Chemistry*, 269(38), 23675-23680.
- Cinco-Moroyoqui, F. J., Rosas-Burgos, E. C., Borboa-Flores, J., & Cortez-Rocha, M. O. (2006). α -Amylase activity of *Rhyzopertha dominica* (coleoptera: Bostrichidae) reared on several wheat varieties and its inhibition with kernel extracts. *Journal of Economic Entomology*, 99(6), 2146-2150.
- Clemente, A., Sonnante, G., & Domoney, C. (2011). Bowman-birk inhibitors from legumes and human gastrointestinal health: Current status and perspectives. *Current Protein and Peptide Science*, 12(5), 358-373.
- Consiglio, A., Grillo, G., Licciulli, F., Ceci, L. R., Liuni, S., Losito, N., et al. (2011). Plantpis - an interactive web resource on plant protease inhibitors. *Current Protein and Peptide Science*, 12(5), 448-454.
- de Azevedo Pereira, R., Nogueira Batista, J. A., Mattar da Silva, M. C., Brilhante de Oliveira Neto, O., Zangrando Figueira, E. L., Valencia Jiménez, A., et al. (2006). An α -amylase inhibitor gene from *Phaseolus coccineus* encodes a protein with potential for control of coffee berry borer (*Hypothenemus hampei*). *Phytochemistry*, 67(18), 2009-2016.
- De Leo F, Bonadé-Bottino M, Ceci LR, Gallerani R, Jouanin L. (2001) Effects of a mustard trypsin inhibitor expressed in different plants on three lepidopteran pests. *Insect Biochemistry and Molecular Biology*, 31(627):593-602.
- De Leo F, Gallerani R. The mustard trypsin inhibitor 2 affects the fertility of *Spodoptera littoralis* larvae fed on transgenic plants (2002) *Insect Biochemistry and Molecular Biology*, 32(5):489-496.
- De Sousa-Majer, M. J., Hardie, D. C., Turner, N. C., & Higgins, T. J. V. (2007). Bean α -amylase inhibitors in transgenic peas inhibit development of pea weevil larvae. *Journal of Economic Entomology*, 100(4), 1416-1422.
- Dias SC, Franco OL, Magalhães CP, de Oliveira-Neto OB, Laumann RA, Figueira EL, Melo FR, Grossi-De-Sá MF. (2005). Molecular cloning and expression of an alpha-amylase inhibitor from rye with potential for controlling insect pests. *Protein Journal*, 24(2):113-123.
- Dias, S. C., da Silva, M. C. M., Teixeira, F. R., Figueira, E. L. Z., de Oliveira-Neto, O. B., de Lima, L. A., et al. (2010). Investigation of insecticidal activity of rye α -amylase inhibitor gene expressed in transgenic tobacco (*Nicotiana tabacum*) toward cotton boll weevil (*Anthonomus grandis*). *Pesticide Biochemistry and Physiology*, 98(1), 39-44.
- Do Nascimento, V. V., Castro, H. C., Abreu, P. A., Elenir, A., Oliveira, A., Fernandez, J. H., et al. (2011). In silico structural characteristics and α -amylase inhibitory properties of

- ric c 1 and ric c 3, allergenic 2S albumins from *Ricinus communis* seeds. *Journal of Agricultural and Food Chemistry*, 59(9), 4814-4821.
- dos Santos, I. S., Carvalho, A. d. O., de Souza-Filho, G. A., do Nascimento, V. V., Machado, O. L. T., & Gomes, V. M. (2010). Purification of a defensin isolated from *Vigna unguiculata* seeds, its functional expression in *Escherichia coli*, and assessment of its insect α -amylase inhibitory activity. *Protein Expression and Purification*, 71(1), 8-15.
- Dunse KM, Stevens JA, Lay FT, Gaspar YM, Heath RL, Anderson MA. (2010) Coexpression of potato type I and II proteinase inhibitors gives cotton plants protection against insect damage in the field. *Proceedings of the National Academy of Sciences USA*, 107(34):15011-15015.
- Farias, L. R., Costa, F. T., Souza, L. A., Pelegrini, P. B., Grossi-de-Sá, M. F., Neto, S. M., et al. (2007). Isolation of a novel *Carica papaya* α -amylase inhibitor with deleterious activity toward *Callosobruchus maculatus*. *Pesticide Biochemistry and Physiology*, 87(3), 255-260.
- Fonseca, F. P. P., Soares-Costa, A., Ribeiro, A. F., Rosa, J. C., Terra, W. R., & Henrique-Silva, F. (2012). Recombinant expression, localization and in vitro inhibition of midgut cysteine peptidase (sl-CathL) from sugarcane weevil, *Sphenophorus levis*. *Insect Biochemistry and Molecular Biology*, 42(1), 58-69.
- Franco, O. L., Melo, F. R., Mendes, P. A., Paes, N. S., Yokoyama, M., Coutinho, M. V., et al. (2005). Characterization of two *Acanthoscelides obtectus* α -amylases and their inactivation by wheat inhibitors. *Journal of Agricultural and Food Chemistry*, 53(5), 1585-1590.
- Franco, O. L., Rigden, D. J., Melo, F. R., Bloch Jr., C., Silva, C. P., & Grossi De Sá, M. F. (2000). Activity of wheat α -amylase inhibitors towards bruchid α -amylases and structural explanation of observed specificities. *European Journal of Biochemistry*, 267(8), 2166-2173.
- Fritig, B., Heitz, T., and Legrand, M. (1998). Antimicrobial proteins in induced plant defense. *Current Opinion in Immunology*, 10, 16-22.
- Gatehouse JA (2011) Prospects for using proteinase inhibitors to protect transgenic plants against attack by herbivorous insects. *Curr Protein Pept Sci*. 12(5):409-16.
- Gatehouse, J. A. (2011). Prospects for using proteinase inhibitors to protect transgenic plants against attack by herbivorous insects. *Current Protein and Peptide Science*, 12(5), 409-416.
- Gomes, M. T. R., Oliva, M. L., Lopes, M. T. P., & Salas, C. E. (2011). Plant proteinases and inhibitors: An overview of biological function and pharmacological activity. *Current Protein and Peptide Science*, 12(5), 417-436.
- Green, T. R., and Ryan, C.A. (1972). Wound-induced proteinase inhibitor in plant leaves: A possible defense mechanism against insects. *Science*, 175, 776-777.
- Halayko, A. J., Hill, R. D., & Svensson, B. (1986). Characterization of the interaction of barley α -amylase II with an endogenous α -amylase inhibitor from barley kernels. *Biochimica et Biophysica Acta / Protein Structure and Molecular*, 873(1), 92-101.
- Ignacimuthu S, Prakash S. (2006). Agrobacterium-mediated transformation of chickpea with alpha-amylase inhibitor gene for insect resistance. *Journal of Biosciences*, 31(3):339-345.

- Ishimoto M, Sato T, Chrispeels MJ, Kitamura K. (1996). Bruchid resistance of transgenic azuki bean expressing seed alpha-amylase inhibitor of common bean. *Entomologia Experimentalis et Applicata*, 79(3):309-315.
- Iulek, J., Franco, O. L., Silva, M., Slivinski, C. T., Bloch Jr., C., Rigden, D. J., et al. (2000). Purification, biochemical characterisation and partial primary structure of a new α -amylase inhibitor from secale cereale (rye). *International Journal of Biochemistry and Cell Biology*, 32(11-12), 1195-1204.
- Jackson, A. O., and Tailor, C. B. (1996) Plant-Microbe Interactions: Life and Death at the Interface. *Plant Cell*, 8, 1651-1668.
- Malek, K., and Dietrich, R. A. (1999) Defense on multiple fronts: How do plants cope with diverse enemies? *Trends Plant Sci.*, 4, 215-219.
- Stotz, H. U., Kroymann, J., and Mitchell-Olds, T. (1999) Plant-insect interactions. *Current Opinion in Plant Biology*, 2, 268-272.
- Johnson R, Narvaez J, An G, Ryan C. (1989) Expression of proteinase inhibitors I and II in transgenic tobacco plants: effects on natural defense against *Manduca sexta* larvae. *Proceedings of the National Academy of Sciences USA*, 86(24):9871-9875.
- Jongsma, M. A., & Beekwilder, J. (2011). Co-evolution of insect proteases and plant protease inhibitors. *Current Protein and Peptide Science*, 12(5), 437-447.
- Kluh, I., Horn, M., Hýblová, J., Hubert, J., Dolečková-Marešová, L., Voburka, Z., et al. (2005). Inhibitory specificity and insecticidal selectivity of α -amylase inhibitor from phaseolus vulgaris. *Phytochemistry*, 66(1), 31-39.
- Liu, Y. -, Cheng, C. -, Lai, S. -, Hsu, M. -, Chen, C. -, & Lyu, P. -. (2006). Solution structure of the plant defensin VrD1 from mung bean and its possible role in insecticidal activity against bruchids. *Proteins: Structure, Function and Genetics*, 63(4), 777-786.
- Nishizawa, K., Teraishi, M., Utsumi, S., & Ishimoto, M. (2007). Assessment of the importance of α -amylase inhibitor-2 in bruchid resistance of wild common bean. *Theoretical and Applied Genetics*, 114(4), 755-764.
- Marshall, J. J., & Lauda, C. M. (1975). Purification and properties of Phaseolamin, an inhibitor of α amylase, from the kidney bean, *Phaseolus vulgaris*. *Journal of Biological Chemistry*, 250(20), 8030-8037.
- Mehrabadi, M., Bandani, A. R., & Saadati, F. (2010). Inhibition of sunn pest, *Eurygaster integriceps*, α -amylases by α -amylase inhibitors (T-AI) from triticale. *Journal of Insect Science*, 10.
- Mehrabadi, M., Bandani, A. R., Saadati, F., & Mahmudvand, M. (2011). α -Amylase activity of stored products insects and its inhibition by medicinal plant extracts. *Journal of Agricultural Science and Technology*, 13(SUPPL.), 1173-1182.
- Mehrabadi, M., Bandani, A. R., Mehrabadi, R., & Alizadeh, H. (2012). Inhibitory activity of proteinaceous α -amylase inhibitors from Triticale seeds against *Eurygaster integriceps* salivary α -amylases: Interaction of the inhibitors and the insect digestive enzymes, *Pesticide Biochemistry and Physiology*, doi: 10.1016/j.pes tpb.2012.01 .008.
- Morton RL, Schroeder HE, Bateman KS, Chrispeels MJ, Armstrong E, Higgins TJV. (2000). Bean alpha-amylase inhibitor 1 in transgenic peas (*Pisum sativum*) provides complete protection from pea weevil (*Bruchus pisorum*) under field conditions. *Proceedings of the National Academy of Sciences USA*, 97(8):3820-3825.

- Mundy, J., Hejgaard, J., & Svendsen, I. (1984). Characterization of a bifunctional wheat inhibitor of endogenous α -amylase and subtilisin. *FEBS Letters*, 167(2), 210-214.
- Mundy, J., Svendsen, I. B., & Hejgaard, J. (1983). Barley α -amylase/subtilisin inhibitor. I. isolation and characterization. *Carlsberg Research Communications*, 48(2), 81-90.
- Ohtsubo, K. -, & Richardson, M. (1992). The amino acid sequence of a 20 kDa bifunctional subtilisin/ α -amylase inhibitor from brain of rice (*oryza sativa* L.) seeds. *FEBS Letters*, 309(1), 68-72.
- Oliva, M. L. V., da Silva Ferreira, R., Ferreira, J. G., de Paula, C. A. A., Salas, C. E., & Sampaio, M. U. (2011). Structural and functional properties of kunitz proteinase inhibitors from leguminosae: A mini review. *Current Protein and Peptide Science*, 12(5), 348-357.
- Oliveira-Neto, O. B., Batista, J. A. N., Rigden, D. J., Franco, O. L., Falcão, R., Frago, R. R., et al. (2003). Molecular cloning of α -amylases from cotton boll weevil, *anthonomus grandis* and structural relations to plant inhibitors: An approach to insect resistance. *Journal of Protein Chemistry*, 22(1), 77-87.
- Pearce, G. (2011). Systemin, hydroxyproline-rich systemin and the induction of protease inhibitors. *Current Protein and Peptide Science*, 12(5), 399-408.
- Pelegri, P. B., Lay, F. T., Murad, A. M., Anderson, M. A., & Franco, O. L. (2008). Novel insights on the mechanism of action of α -amylase inhibitors from the plant defensin family. *Proteins: Structure, Function and Genetics*, 73(3), 719-729.
- Roberts, T.H.; Hejgaard, J. (2008). Serpins in plants and green algae. *Functional and Integrative Genomics*, 8(1), 1-27.
- Salas, C.E.; Gomes, M.T.; Hernandez, M.; Lopes, M.T. (2008). Plant cysteine proteinases: evaluation of the pharmacological activity. *Phytochemistry*, 69(12), 2263-2269.
- Sarmah BK, Moore A, Tate W, Molvig L, Morton RL, Rees DP, Chiaiese P, Chrispeels MJ, Tabe LM, Higgins TJV.(2004). Transgenic chickpea seeds expressing high levels of a bean alpha-amylase inhibitor. *Molecular Breeding*, 14(1):73-82.
- Schaller, A. (2004) A cut above the rest: the regulatory function of plant proteases. *Planta*, 220(2), 183-197.
- Schimoler-O'Rourke, R., Richardson, M., & Selitrennikoff, C. P. (2001). Zeamatin inhibits trypsin and α -amylase activities. *Applied and Environmental Microbiology*, 67(5), 2365-2366.
- Schroeder HE, Gollasch S, Moore A, Tabe LM, Craig S, Hardie DC, Chrispeels MJ, Spencer D, Higgins TJV (1995). Bean alpha-Amylase Inhibitor Confers Resistance to the Pea Weevil (*Bruchus pisorum*) in Transgenic Peas (*Pisum sativum*). *Plant Physiology*, 107(4), 1233-1231.
- Shade RE, Schroeder HE, Pueyo JJ, Tabe LM, Murdock LL, Higgins TJV, Chrispeels MJ. (1994) Transgenic Pea-seeds expressing the alpha-amylase inhibitor of the common bean are resistant to bruchid beetles. *Bio-Technology*, 12(8):793-796.
- Silva, D. P., Casado-Filho, E. L., Corrêa, A. S. R., Farias, L. R., Bloch Jr., C., Grossi De Sá, M. F., et al. (2007). Identification of an α -amylase inhibitor from *pterodon pubescens* with ability to inhibit cowpea weevil digestive enzymes. *Journal of Agricultural and Food Chemistry*, 55(11), 4382-4387.

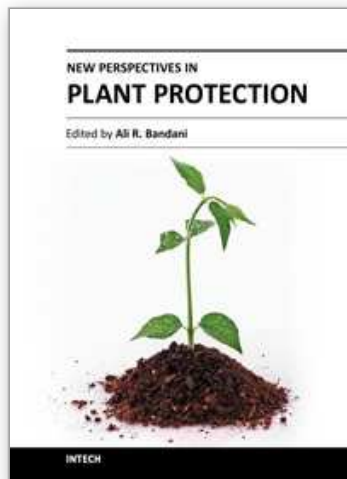
- Silva, E. M., Valencia, A., Grossi-de-Sá, M. F., Rocha, T. L., Freire, E., de Paula, J. E., et al. (2009). Inhibitory action of cerrado plants against mammalian and insect α -amylases. *Pesticide Biochemistry and Physiology*, 95(3), 141-146.
- Simoni Campos Dias, Maria Cristina Mattar da Silva a, Fábíola R. Teixeira a, Edson Luis Zangrando Figueira a,d, Osmundo Brilhante de Oliveira-Neto a,d, Loiane Alves de Lima c, Octávio Luiz Franco c,e, Maria Fátima Grossi-de-Sa (2010) Investigation of insecticidal activity of rye α -amylase inhibitor gene expressed in transgenic tobacco (*Nicotiana tabacum*) toward cotton boll weevil (*Anthonomus grandis*) *Pesticide Biochemistry and Physiology*, 98(1), 39-44.
- Soares-Costa, A., Dias, A. B., Dellamano, M., de Paula, F. F. P., Carmona, A. K., Terra, W. R., et al. (2011). Digestive physiology and characterization of digestive cathepsin L-like proteinase from the sugarcane weevil *Sphenophorus levis*. *Journal of Physiology*, 57(4), 462-468.
- Solleti, S. K., Bakshi, S., Purkayastha, J., Panda, S. K., & Sahoo, L. (2008). Transgenic cowpea (*Vigna unguiculata*) seeds expressing a bean α -amylase inhibitor 1 confer resistance to storage pests, Bruchid beetles. *Plant Cell Reports*, 27(12), 1841-1850.
- Svensson, B., Asano, K., Jonassen, I., Poulsen, F. M., Mundy, J., & Svendsen, I. (1986). A 10 kD barley seed protein homologous with an α -amylase inhibitor from indian finger millet. *Carlsberg Research Communications*, 51(7), 493-500.
- Turrà, D., & Lorito, M. (2011). Potato type I and II proteinase inhibitors: Modulating plant physiology and host resistance. *Current Protein and Peptide Science*, 12(5), 374-385.
- United States Environmental Protection Agency (2009) *Bacillus thuringiensis* Cry1Ab delta-endotoxin protein and the genetic material necessary for its production (via elements of vector pZO1502) in event Bt11 Corn (OECD unique identifier: SYN-BTØ11-1)(006444) and *Bacillus thuringiensis* Vip3Aa20 insecticidal protein and the genetic material necessary for its production (via elements of vector pNOV1300) in event MIR162 maize (OECD unique identifier: SYN-IR162-4)(006599) and modified Cry3A protein and the genetic material necessary for its production (via elements of vector pZM26) in event MIR604 corn (OECD unique identifier: SYN-IR6Ø4-5)(006509). Fact sheet 006599-006444-006509. Available at: http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_006599-006444.html. Accessed February 2, 2010.
- Valencia-Jiménez, A., Arboleda Valencia, J. W., & Grossi-De-Sá, M. F. (2008). Activity of α -amylase inhibitors from *Phaseolus coccineus* on digestive α -amylases of the coffee berry borer. *Journal of Agricultural and Food Chemistry*, 56(7), 2315-2320.
- Volpicella, M., Leoni, C., Costanza, A., de Leo, F., Gallerani, R., & Ceci, L. R. (2011). Cystatins, serpins and other families of protease inhibitors in plants. *Current Protein and Peptide Science*, 12(5), 386-398.
- Waniek, P. J., Pacheco Costa, J. E., Jansen, A. M., Costa, J., & Araújo, C. A. C. (2012). Cathepsin L of *Triatoma brasiliensis* (Reduviidae, Triatominae): Sequence characterization, expression pattern and zymography. *Journal of Physiology*, 58(1); 178-187.
- Wisessing, A., Engkagul, A., Wongpiyasatid, A., & Choowongkamon, K. (2010). Biochemical characterization of the α -amylase inhibitor in mungbeans and its application in

inhibiting the growth of *callosobruchus maculatus*. *Journal of Agricultural and Food Chemistry*, 58(4), 2131-2137.

Zoccatelli, G., Pellegrina, C. D., Mosconi, S., Consolini, M., Veneri, G., Chignola, R., et al. (2007). Full-fledged proteomic analysis of bioactive wheat amylase inhibitors by a 3-D analytical technique: Identification of new heterodimeric aggregation states. *Electrophoresis*, 28(3), 460-466.

IntechOpen

IntechOpen



New Perspectives in Plant Protection

Edited by Prof. Ali R. Bandani

ISBN 978-953-51-0490-2

Hard cover, 246 pages

Publisher InTech

Published online 11, April, 2012

Published in print edition April, 2012

Crop losses by pests (insects, diseases and weeds) are as old as plant themselves but as agriculture are intensified and cropping patterns including the cultivation of high yielding varieties and hybrids are changing over time the impact of the pests becoming increasingly important. Approximately less than 1000 insect species (roughly 600-800 species), 1500 -2000 plant species, numerous fungal, bacterial and nematode species as well as viruses are considered serious pests in agriculture. If these pests were not properly controlled, crop yields and their quality would drop, considerably. In addition production costs as well as food and fiber prices are increased. The current book is going to put Plant Protection approaches in perspective.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Mohammad Mehrabadi, Octavio L. Franco and Ali R. Bandani (2012). Plant Proteinaceous Alpha-Amylase and Proteinase Inhibitors and Their Use in Insect Pest Control, *New Perspectives in Plant Protection*, Prof. Ali R. Bandani (Ed.), ISBN: 978-953-51-0490-2, InTech, Available from: <http://www.intechopen.com/books/new-perspectives-in-plant-protection/plant-proteinaceous-amylase-and-proteinase-inhibitors-and-their-use-in-insect-pest-control>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen