INTRODUCTION

The recent development of refined molecular tools to study gene structure and function, as well as the availability of genetic transformation methods for all kinds of organisms, including plants, has lent new vigor to the investigation of plant defense mechanisms, a subject with a long and distinguished tradition.

A variety of approaches are being followed by molecular biologists to analyze plant responses to pathogens and pests. Three of these emerge as predominant: i) From disease to relevant molecules, a strategy that involves challenging a plant with a pathogen or pest, identifying the plant genes that are switched on in response to the challenge, and attempting to correlate the functional properties of at least some of these genes with a defense role. ii) From disease resistance to relevant molecules, an approach based on finding cosegregation of a resistance gene and certain molecular features in a near-isogenic background. iii) From molecules with toxic or deterrent properties to enhanced resistance, an approximation which implies the construction of an agronomic trait out of the known activities of appropriate molecules and their corresponding genes.

It is not pertinent here to discuss the relative merits of these strategies, which are not mutually exclusive. My aim is rather to examine to what extent they have allowed the identification of possible defense molecules in barley and related species. Because of circumstantial and/or objective reasons, monocots, in general, and barley, in particular, have not been the plant systems where some of the original studies have been carried out. However, for obvious reasons a significant part of recent interest is focusing on them. Plant defense mechanisms are still far from being elucidated (see Bowles 1990), so this non-comprehensive review will have the more modest objective of summarizing some relevant information concerning possible defense molecules in barley and its close relatives.

TYPES OF MOLECULES

Types of molecules whose in vitro properties and/or physiological behaviour suggest possible involvement in defense and are present in barley or in closely-related species are listed in Table I. Most of these, but not all, are proteins that show toxic or inhibitory activities towards heterologous systems or whose genes are induced when challenged with pathogens or pests.

Pathogenesis-related (PR) proteins induced in plants infected with pathogens
Table I. Possible defense molecules in barley

<table>
<thead>
<tr>
<th>Type</th>
<th>Variants</th>
<th>Active against</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitinases</td>
<td>C</td>
<td>fungi</td>
<td>Endosperm</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>&quot;</td>
<td>Alcurone</td>
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<tr>
<td></td>
<td>K</td>
<td>&quot;</td>
<td>Cell suspension</td>
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<tr>
<td></td>
<td>PR3</td>
<td></td>
<td>Leaves</td>
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<tr>
<td>Glucanases</td>
<td>GI-GVI</td>
<td>fungi</td>
<td>Endosperm</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Leaves</td>
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<td>Cell suspension</td>
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<td></td>
<td></td>
<td></td>
<td>Roots</td>
</tr>
<tr>
<td>RIP</td>
<td>&gt;1</td>
<td>ribosomes fungi</td>
<td>Endosperm</td>
</tr>
<tr>
<td>Inhibitors</td>
<td>BASI</td>
<td>subtilisin</td>
<td>Endosperm</td>
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<tr>
<td></td>
<td></td>
<td>α-amylase endogenous</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CI-1</td>
<td>chymotrypsin</td>
<td>Endosperm</td>
</tr>
<tr>
<td></td>
<td>CI-2</td>
<td>subtilisin</td>
<td></td>
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<tr>
<td>PR5</td>
<td>?</td>
<td></td>
<td>Leaves</td>
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<tr>
<td></td>
<td></td>
<td>monomeric, Heterologous</td>
<td></td>
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<td></td>
<td></td>
<td>dimeric, α-amylase (insects)</td>
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<td></td>
<td></td>
<td>tetrameric</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>trypsin</td>
<td>Endosperm</td>
</tr>
<tr>
<td>Thionin 1</td>
<td>α</td>
<td>bacteria</td>
<td>Endosperm</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>and fungi</td>
<td></td>
</tr>
<tr>
<td>Thionin II</td>
<td>DB4</td>
<td>bacteria</td>
<td>Leaves</td>
</tr>
<tr>
<td></td>
<td>DG3</td>
<td>and fungi</td>
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<tr>
<td></td>
<td>DG4</td>
<td></td>
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<tr>
<td>Thionin V</td>
<td>TTHV</td>
<td>?</td>
<td>Endosperm</td>
</tr>
<tr>
<td>PR Proteins</td>
<td>PR1 &amp; others</td>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td>Lectins</td>
<td>?</td>
<td></td>
<td>Germ ?</td>
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<tr>
<td>Hydroxamic acids</td>
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<td></td>
<td>Ubiquitous</td>
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or treated with chemicals, are extractable at low pH and predominantly appear in
the intracellular spaces (see Van Loon, 1985). These proteins have been most
intensively studied in dicots and only recently have they been identified in
monocots and, more specifically, in barley, where proteins corresponding to PR1
and PR5 from tobacco have been found (White et al. 1987; Bryngelson et al. 1988;
Bryngelson and Green 1989). Up to ten PR proteins have been isolated from
maize, four of which have been identified as chitinases and two as 1,3-β-
glucanases (Nasser et al. 1988, 1990). These hydrolases have been reported to
inhibit fungal growth in vitro (Schlumbaum et al. 1986; Mauch et al. 1988). Barley
and wheat have both chitinases (Roberts and Selitrennikoff 1986; Leah et al. 1987,
1991; Broekaert et al. 1988; Swegle et al. 1989; Jacobsen et al. 1990; Ride and Barber
1990; Kragh et al. 1991) and 1,3-β-glucanases (Ballance and Svendsen 1988; Høj
et al. 1988, 1989; Sock et al. 1990; Kragh et al. 1991; Leah et al. 1991) in various
tissues, specially in endosperm, and under different physiological situations.

A complex class of possible defense-related proteins is represented by plant
proteinaceous inhibitors of proteases and α-amylases of heterologous systems (see
García-Olmedo et al. 1987). These were classified into 11 families or types of which,
more than half were represented in cereals. The activity in vitro and in vivo of
some of these inhibitors versus insect enzymes and whole insects has been
recently reviewed (García-Olmedo et al. 1991).

The thionins are 5 kDa polypeptides, which are cysteine-rich and whose
toxicity to plant pathogens was reported long ago (Fernández de Caleya et al. 1972).
Different structural types and several genetic variants of each have been
described in barley, as well as in other taxa (see García-Olmedo et al. 1989, 1991,
1992). A 30 kDa ribosome-inactivating protein (RIP) from barley, related to ricin
and tritin, has antifungal activity (Asano et al. 1986; Leah et al. 1991).

Some possible defense proteins characterized in wheat have not yet been
identified in barley. This is the case, for example, of wheat germ agglutinin
(Smith and Raikhel 1989; Huesing et al. 1991) and a pathogen-induced protein
which is homologous to glutathione-S-transferase (Dudler et al. 1991).

No evidence of phytoalexins exhibiting a defense function in cercáis is
available, but in contrast, lignification seems to play a key role in the
hypersensitive reaction (Moerschbacher et al. 1990). Thus, specific suicidal
inhibitors of the lignification pathway, applied prior to inoculation of resistant
wheat with stem rust, decreased the frequency of necrotic host cells and led to
increased fungal growth (Moerschbacher et al. 1990).

Among non-protein defense-related molecules in cereals, hydroxamic acids
have received particular attention (see Niemeyer 1988). These compounds are
active against pathogens and other organisms, but an unequivocal link to defense
mechanisms in vivo is still lacking (Niemeyer 1988).

Cloning of cDNAs corresponding to mRNAs induced during infection is an
approach which is actively pursued at present, as, for example, in the resistant
barley/powdery mildew interaction (Davidson et al. 1987).

The more relevant types of molecules alluded to above will be discussed in
the following paragraphs, with special emphasis on their plant-defense properties
in vitro and/or in vivo.
CHITINASES AND 1,3-β-GLUCANASES

A 28 kDa protein from barley endosperm (protein C) with antifungal properties (Roberts and Selitrennikoff 1986) was subsequently found to be an endochitinase (Leah et al. 1987). A closely similar one was identified in barley aleurone (Swegle et al. 1989), which was later identified as the antifungal basic chitinase T. It is a 33 kDa protein with a 23 amino acid extension at the N-terminal domain that was not present in chitinase C and was homologous (73% identity) with the B domain of wheat germ lectin and the N-terminal of a chitinase from bean leaves (Jacobsen et al. 1990). What seems to be an isoform of chitinase C, designated CHI26 has been recently reported by Leah et al. (1991). Suspension cultures of barley secrete chitinases T and C and a third chitinase, designated K, which is also present in the barley grain (Kragh et al. 1991). The first chitinase, PR3, has been described in barley leaves (Bryngelson et al. 1991), and four major forms have been recently purified from wheat leaves (Ride and Barber 1990). Two 1,3-β- endoglucanases, designated GI and GII, have been purified and their corresponding cDNAs cloned from barley seeds (Hoj et al. 1988, 1989; Ballance and Svendsen 1988; Leah et al. 1991). One of them, GII, has been identified among secreted proteins in suspension cultures (Kragh et al. 1991). More recently, up to six 1,3-β-endoglucanases (GI-GVI) have been identified and their differential expression pattern has been studied (Slakeski et al. 1991). No investigation of their induction in barley leaves seems to be available, but these enzymes have been shown to be elicited in wheat leaves both abiotically and by stem rust infection (Sock et al. 1990).

In line with previous observations with other chitinases and 1,3-β-glucanases (Mauch et al. 1988), Leah et al. (1991) have shown synergism between the two types of barley enzymes in their inhibitory activity against fungal pathogens in vitro. Furthermore, they have shown synergism between these enzymes and the ribosome-inactivating protein.

PROTEINACEOUS INHIBITORS OF PROTEASES AND α-AMYLASES

Inhibitor families

The Kunitz (STI) family includes inhibitors of serine proteinases and endogenous α-amylases (see García-Olmedo et al. 1987) and is represented in barley by an inhibitor, designated BASI, which inhibits subtilisin and type-2 endogenous α-amylase (Mundy et al. 1984; Svendsen et al. 1986).

Double-headed and single-headed trypsin inhibitors from wheat germ (Odani et al. 1986) are homologous to the well characterized double-domain Bowman-Birk inhibitors from the Leguminosae, which inhibit trypsin and chymotrypsin. Occurrence of these inhibitors in barley germ has not been demonstrated, but can be considered as likely.

Two inhibitors of barley endosperm, CI-1 and CI-2, which are homologous to the potato inhibitor I, have been well characterized (Jonassen and Svendsen 1982). Both of them inhibit chymotrypsin and subtilisin, but CI-1 has a single reactive
site for the two enzymes and CI-2 has one site for chymotrypsin and two for subtilisin.

Bryngelson and Green (1989) have characterized a pathogenesis-related, thaumatin-like protein, designated PR-5 which is induced in barley leaves when challenged with an incompatible race of mildew. This protein is homologous with PR-5 from tobacco and is also related to an inhibitor of porcine pancreatic α-amylase from Eleusine coracana (Campos and Richardson 1984) and to the sweet protein thaumatin II. A second barley protein, designated PAPI, is more distantly related (Svensson et al. 1986).

The cereal trypsin/α-amylase inhibitor family is perhaps the most diversified among those in barley (Table I), as it includes both trypsin inhibitors and the subunits of monomeric, dimeric and tetrameric inhibitors of heterologous α-amylases (see García-Olmedo et al. 1987, 1991, Sanchez-Monge et al. 1988). These inhibitors are encoded by a multigene family which is dispersed over several chromosomes in both wheat and barley.

Defense properties of the inhibitors

It is difficult to speculate about the natural targets for the rich arsenal of potential weapons represented by the above inhibitors. The last family's activity against the α-amylases from insects suggested long ago its possible role in pest control. Thus, monomeric and dimeric α-amylase inhibitors show different specificity versus the α-amylases from human saliva and from the digestive tract of the insect Tenebrio molitor. The tetrameric inhibitors seem to be active against the α-amylase from the insect but not from the salivary (Sanchez-Monge et al. 1986; Gomez et al. 1989). Furthermore, different insect α-amylases are discriminated by the inhibitors: i.e. the enzyme from T. molitor is more sensitive to monomeric than to dimeric types and the opposite is true for the origine from Leptinotarsa decemlineata (colorado potato beetle); still for other insect enzymes, both types of inhibitors are about equally effective (Gutierrez et al. 1990).

Insects that are able to feed on cereal endosperm have unusually high levels of α-amylase (Silano et al. 1975; Gutierrez et al. 1990). Other insects, such as Callosobruchus maculatus are sensitive to low inhibitor concentrations in the diet (Gatehouse et al. 1986). More recently, transgenic tobacco plants carrying chimeric genes encoding α-amylase and trypsin inhibitors from this family have been found to be lethal to Agrotis ipsilon and Spodoptera littoralis in a leaf-disc assay (Carbonero et al. unpublished).

THIONINS

Types present

The thionins are polypeptides of about 5 kDA, which have three or four disulphide bridges and are toxic to plant pathogens (García-Olmedo et al. 1989, 1991a,b). Out of five structural thionin types into which all the known sequences of this family can be classified (García-Olmedo et al. 1992), three of them (I, II, V) are represented by one or more genetic variants in barley and wheat. Type I
corresponds to the original endosperm thionins. Thionins of this type have four disulphide bridges and are highly basic (see García-Olmedo et al. 1992). Type II correspond to the leaf thionins, which are structurally very similar to those of type I (Gausing 1987; Bohlman and Apel 1987). Type V thionins have three disulphide bridges and very few charged amino acids. They are also in the endosperm (A. Castagnaro, unpublished).

Thionins of types I and V accumulate in endosperm during the first half of its developmental period, while those of type II are synthesized in ethiolated leaves or in green leaves under stress conditions (Bohlman et al. 1988; Ebrahim-Nesbat et al. 1989).

Defense properties of thionins

The toxicity of thionins towards different kinds of organisms and to cells in culture has been investigated for several decades (see García-Olmedo et al. 1991a,b). Plant pathogenic bacteria of the genera Pseudomonas, Xanthomonas, Agrobacterium, Erwinia and Corynebacterium are sensitive to thionin (Fernandez de Caleya et al. 1972 and unpublished). In a recent survey, sensitivity of fungal pathogens to pure genetic variants was in the 10^{-6} M-10^{-5} M range (García-Olmedo et al. 1991a,b; Molina and Fraile, unpublished). We have obtained transgenic tobacco plants constitutively expressing the α-thionin gene from barley. These plants show enhanced resistance to the bacterial pathogens Pseudomonas solanacearum and P. syringae pv. tabaci.

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