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Chitinase as a possible resistance factor for higher plants

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Summary. Virulence and resistance may act on the same biochemical mechanisms. Because *Erwinia*-virulence on potato depends on the lysis of cell walls of the host, resistance may depend on the lysis of cell walls of the parasite. An example is given with yellow rust on wheat.

Key words: Resistance – Genetic engineering – Yellow rust – Wheat – Chitinase

Introduction

If a plant is damaged by a parasite, we call it virulence; if a parasite is damaged by a plant, we call it resistance. Both of these definitions have been made by man, not by nature. For instance, resistant truffles are known to exist. So in searching for resistance-factors it seems useful to look at every different kind of factors by which organisms influence each other.

Erwinia carotovora (Jones) Bergey et al. is a bacterium which, in producing pectinase, damages *Solanum tuberosum* L. (potato) tubers by lysing its cell walls (Mount et al. 1970). Because we have here a parasite which has an advantage, it can damage the cell walls of the host, it might be useful by analogy to look at a system where the host will lyse the cell walls of the parasite, assuming that in this case the host will become resistant. While the cell walls of plants consist of cellulose, those of the fungi consist of chitin. Accordingly chitinase might be a useable resistance factor for genetic engineering. As a first step in testing this hypothesis, the influence of chitinase on the infection rate of yellow rust on wheat was tested.

Material and methods

Wheat (*Triticum aestivum* L. cv. 'Ural') was germinated in 8 cm pots (5 seeds per pot) and then treated with a 1.5% solution of milstem. One week later the same plants were treated with a 1.5% solution of maleic acid hydrazide in order to prevent mildew infection and to reduce plant growth (Grunewaldt-Stöcker 1981). Nine days after sowing the seeds, at growth stage 12 (Zadoks et al. 1974) the plants were inoculated with freshly harvested spores of yellow rust (*Puccinia striifomis* West), a mixture of different pathotypes. Inoculation was done with a lancet, which was moistened with (a) tap water or (b) 0.625%, (c) 1.25% or (d) 2.5% solution of chitinase in tap water.

After inoculation the plants were grown at 100% relative humidity (RH) 24 h and subsequenty at 80–85% RH and 15 °C for 25 days. The yellow rust growth was then scored on a 1–9 scale (1 = no infection, 9 = severe infection).

Results

The data of the experiment are presented in Table 1. Plants without or with only a mild infection occur much more frequently in the chitinase treated variants than in the controls. The distribution of the controls differs significantly from those of the others (χ^2 [ab] = 26.4, [ac] = 19.6, [ad] = 31.9, all 8 df). None of the chitinase treated variants differ significantly from each other. These results implicate a reduction of the yellow rust infection by the enzyme chitinase.

Discussion

For genetic engineering of resistances the identification of resistance genes is the first step which must be solved. With classical methods of analysing segregation ratios, only genes acting within a species can be

Table 1. Frequency of plants in score classes for yellow rust influenced by chitinase during inoculation

Variant	Score class									
	1	2	3	4	5	6	7	8	9	S
[a] tap water	6	12	15	15	12	6	1	1	17	85
[b] tap water + 0.625% chitinase	20	27	12	9	6	0	1	1	8	84
[c] tap water + 1.25% chitinase	25	12	15	7	5	3	2	1	12	82
[d] tap water + 2.50% chitinase	27	23	8	8	2	3	0	0	13	84

detected. Because many parasites are highly specialized, it can not be assumed that such genes also act in other species. A gene transfer inside of the original species can be done by classical breeding methods as genetic engineering will surely be too expensive.

The present model offers a system for detecting resistant genes in unrelated species. The reduction of the infection rate of yellow rust on wheat demonstrated that chitinase may act as a resistance-factor. The argument that chitinase cannot be such a factor, because the fungus itself produces chitinase, is not realistic. The potato also produces pectinases, nevertheless, it is attacked by the pectinases of *Erwinia.*

If chitinase is a resistance factor, it offers many advantages to molecular biology and genetic engineering:

1) The enzyme chitinase is available and sold commercially (Sigma Chemicals).
2) Organisms, from which the chitinase gene can be isolated, are known (i.e. *Streptomyces griseus* Krainsky).
3) These organisms are procaryotes and can easily be handled.
4) Methods for gene identification and cloning are available.
5) It is known that procaryotic genes can be transfered to higher plants and that they can produce functional enzymes (Otten et al. 1981).
6) The resistance factor is an enzyme; one can hope that this enzyme will not affect secondary metabolism and does not delete any important products.
7) Higher plants do not contain chitin, therefore chitinase should not be toxic to higher plants.
8) Gene regulation is not neccessary, the chitinase may act during all developmental stages of the plant.
9) It may be assumed that the gene should act in many species, both dicots and monocots.
10) It may be assumed that chitinase may act not only against fungi, but also against arthropods and other chitin containing organisms.

The present theoretical model offers a possibility for the transfer of a resistance gene. However the decision if chitinase is really a resistance gene can only be given after a successful transfer to a higher plant.

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References

Grunewaldt-Stöcker G (1981) Untersuchungen zur Rassenanalyse von *Puccinia hordei* Otth und zur Braunrostresistenz der Gerte. PhD Thesis, Hannover

Mount MS, Bateman DF, Basham HG (1970) Induction of electrolyte loss, tissue maceration, and cellular death of potatoe tissue by an endopolygalacturonate trans-eliminase. Phytopathology 60:924–931

Otten L, de Greve H, Hernalsteens JP, Montagu van M, Schieder O, Straub J, Schell J (1981) Mendelian transmission of genes introduced into plants by the Ti plasmids of *Agrobacterium tumefaciens.* Mol Gen Genet 183: 209–213

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421