A Fungal Elicitor of the Resistance Response in Wheat

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An elicitor of the lignification response in wheat leaves was isolated from the germ-tube walls of wheat stem rust. The active compound causes metabolic changes typically correlated with the resistance response, *i.e.* the formation of lignin or lignin-like polymers in affected epidermal and mesophyll cells and the increased activities of enzymes involved in the phenylpropanoid-pathway.

Defense reactions in plants against pathogens can be artificially induced by a wide range of abiotic and biotic factors commonly referred to as elicitors [1, 2]. These have been used as tools in the study of plant resistance phenomena. Of special interest are those elicitors which have been isolated from the pathogen itself. Considerable evidence supports the view that fungal elicitors are involved in the induction of resistance responses *in vivo* [3]. In accordance with the gene-for-gene hypothesis in host-parasite interactions several authors have discussed elicitors as products of genes for avirulence. One regards the elicitors as interacting in one way or another with the products of corresponding genes for resistance of the host, thus specifying the recognition process as a prerequisite of the active defense [4, 5].

In highly resistant wheat cultivars the resistance response against *Puccinia graminis* is the hypersensitive cell death. The resistance is seen through a rapid necrosis of those leaf cells that have been penetrated by haustoria. The cell death is correlated with an extensive synthesis of lignin or lignin-like polymers and of callose [6, 7].

We now succeeded in isolating an elicitor-active component from the germ-tube walls of *Puccinia graminis* f. sp. *tritici* by aqueous extraction. Applying this elicitor to wheat leaves the same symptoms emerge as in rust-infected highly resistant leaves. Furthermore, the rust elicitor stimulates the following enzymes: phenylalanine ammonia-lyase (PAL), 4-coumarate: CoA ligase (4-Cl), cinnamylalcohol-dehydrogenase (CAD) and peroxidase (Table I).

Table I. Enzyme activities after elicitor-treatment of wheat leaves.

	PAL activity [%]	4-Cl activity [%]	CAD activity [%]	PO activity [%]
wheat leaves carrying the *Sr5* gene	3825	758	584	352
wheat leaves carrying the *sr5* gene	2284	769	580	264

Increased activities of enzymes involved in the biosynthesis of lignin after injection of an aqueous solution containing 0.04% (glucose equivalents) of the elicitor from *P. graminis* f. sp. *tritici* (race 32). The elicitor was injected into the intercellular spaces of 7-day-old primary leaves of the near isogenic wheat line marquis carrying either the *Sr5* gene for resistance or the corresponding *sr5* gene for susceptibility.

Enzyme activities are expressed as percentage of untreated controls. Injection of water alone did not lead to increased enzyme activities.

These enzymes are involved in lignin synthesis and show increased activities in resistant wheat cultivars after inoculation with uredospores.

With regard to the specificity the elicitor preparation was tested on two near isogenic wheat lines (background marquis) either carrying the *Sr5* gene for resistance or the allel imparting susceptibility to race 32 of *Puccinia graminis tritici*. When isolated protoplasts are treated with the elicitor they show the hypersensitive-like cell death as described by Doke and Tomiyama [8]. Protoplasts from cultivars carrying the *Sr5* gene for resistance react faster than those carrying the gene for susceptibility (Fig. 1).

There is evidence for another differential effect. In both wheat lines PAL activity increases with rising elicitor concentration, but wheat plants carrying the *Sr5* gene react more sensitive compared to plants carrying the *sr5* gene (Table I). Since PAL has been proposed to play a regulatory role in the metabolism of phenolics (including lignin) [9], a differential

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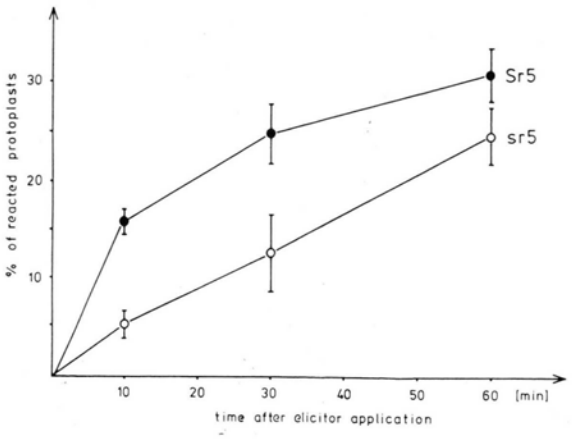

Fig. 1. The effect of the elicitor from germ-tube walls of *Puccinia graminis* f. sp. *tritici* on wheat leaf protoplasts (background marquis) carrying either the *Sr5* gene for resistance or the *sr5* gene for susceptibility. The reaction mixture contained 10^5 protoplasts and 15 µg elicitor in 200 µl solution [4].
The protoplasmic aggregation of protoplasts followed by exolysis of the aggregate leaving a ghost of spherical membrane behind (hypersensitive-like cell death [8]) was determined by microscopy at different times after elicitor application.
Protoplasts from 7-day-old cultivars carrying the *Sr5* gene for resistance react faster than those carrying the *sr5* gene imparting susceptibility.
Each point is the mean value of 10 replications. At each time of the experiment protoplasts carrying the *Sr5* gene reacted significantly more sensitive than protoplasts carrying the *sr5* gene (t-test, $p = 0.05$; (±) standard deviation).
●——● Marquis carrying the *Sr5* gene for resistance;
○——○ marquis carrying the *sr5* gene for susceptibility.

stimulation of this enzyme may also be of importance in the natural interaction.

As determined by ultrafiltration the molecular mass of the active compound is approximately 100000.

Analysis by gas liquid chromatography shows that the elicitor fraction is composed mainly of glucose with small amounts of mannose and galactose. The protein content is approximately 30%. Treatments with periodate and pronase support the view that the carbohydrate moiety bears the active part of the molecule. The elicitor preparation was subjected to 1 or 2 dimensional discontinuous polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS-PAGE). ConA-peroxidase overlay of electroblots shows the presence of glycoproteins with terminal α-glucose or α-mannose whereas an overlay with soybean agglutinin (SBA) labeled peroxidase only shows very weak binding. The results suggest that the rust elicitor is a glycoprotein with ConA binding activity.

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