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About Changes in the Chromatin Structure after Resistance Induction in *Hordeum vulgare* L.

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With 2 figures

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Abstract

The application of the three resistance inducers Trigonelline (N-Methyl-nicotinic acid), Isonicotinic acid methyl ester and the culture filtrate of *Bacillus subtilis* (B 50) to barley resulted in a reduction of infection with powdery mildew up to 65 %. When the isolated high molecular-weight DNA was hydrolized to single bases the genomic DNA methylation of induced resistant plants was reduced between 3.1 % and 4.8 % compared to the control. On the basis of the given results the hypothesis that inducers could change the DNA structure is discussed considering further information from literature about gene regulation.

Zusammenfassung

Zur möglichen Veränderung der Chromatinstruktur nach Resistenzinduktion in *Hordeum vulgare* L.

Die Resistenzinduktoren Trigonellin (N-Methylnicotinsäure), Isonicotinsäure-Methylester und das Kulturfiltrat von einer *Bacillus subtilis*-Flüssigkultur (B 50) konnten den Befall von Gerste mit Echtem Mehltau bis zu 65 % reduzieren. Zwei Tage nach Applikation der Induktoren wies die DNA der induziert resistenten Pflanzen einen um 3,1 % bis 4,8 % reduzierten Methylierungsgrad im Vergleich zur Kontrolle auf. Die Ergebnisse stützen die Hypothese, daß die getesteten Resistenzinduktoren die Chromatinstruktur verändern können, so daß eine veränderte Genregulation die Folge sein könnte. Die Ergebnisse werden im Rahmen der vorliegenden Literatur diskutiert.

Trigonelline (KRASKA, unpublished), Isonicotinic acid methyl ester (METRAUX *et al.* 1991) and the culture filtrate from *Bacillus subtilis* (SCHÖNBECK *et al.* 1980) induce a higher degree of resistance of plants towards obligate biotrophic

pathogens. The hormone-like character of Trigonelline (TRAMONTANO *et al.* 1981) suggests that the induction of resistance is possibly based on a modified gene regulation. TAGUCHI *et al.* (1989) discuss the effects of Trigonelline on DNA metabolism via enzyme inhibition. Also, there might be certain analogies to the epigenetic mechanisms described by HOLLIDAY (1987) for animal systems affecting transcription. In this respect the role of DNA methylation and its possible function in gene regulation and activation are mainly considered in the thorough discussion of ADAMS and BURDON (1985), as well as the effects of methylation inhibitors (e.g. 5-Azacytidine or Ethionine) or pathogens and environmental factors on the level of DNA methylation. It can be summarized from the literature that gene activation or reactivation is correlated with a hypomethylation of the DNA and a hypersensitivity of chromatin towards DNaseI (KLAAS and AMASINO 1989).

The aim of this work was to find out, if the tested resistance inducers in the system barley—powdery mildew could possibly alter the chromatin structure in such a way, that this modification could be observed in a different level of genomic DNA methylation two days after resistance induction as it could be found after a treatment with DNA methylation inhibitors.

Materials and Methods

Barley (*Hordeum vulgare* L.) plants (cv. Mammut) were cultivated in pots and the primary leaves were treated with the agents or with water as a control. Two days after application of the substances the plants were inoculated with *Erysiphe graminis* f. sp. *hordei;* 7 days later the infection density was evaluated as colonies per primary leaf.

The culture filtrate of *Bacillus subtilis* was applied directly to the plants, whereas Trigonelline and Isonicotinic acid methyl ester were suspended in water; the used concentration are given in Results and Discussion.

The extraction procedure of the chromatin and the purification of the DNA from the primary leaves of barley plants 2 days after treatment followed the methods of STEINMÜLLER and APEL (1986), agarose gel-electrophoresis was carried out as described in MANIATIS *et al.* (1989).

The preparation of DNA bases for determination of m^5-cytosine (m^5 Cyt) was done following the method of KLAAS *et al.* (1989). DNA bases were separated on a Beckmann-HPLC-system with a reversed phase column (LiChrospher 100 RP-18, 5 μm, in LiChroCART 125-4, Merck, Germany) by using a linear gradient from 0 % to 10 % buffer B in 15 min at a flow rate of 1 ml/min. The buffers were: 20 mM KH_2PO_4, pH 5.4/NaOH; 5 mM hexanesulfonic acid; 0.7 % methanol (buffer A) or 20 % methanol (buffer B) respectively. The bases were detected at 280 nm and $\%m^5$ Cyt was calculated as $\%m^5 \text{ Cyt} = m^5 \text{ Cyt} \times 100/(m^5 \text{ Cyt} + \text{Cyt})$ after peak integration. The identification of the peaks were done with base standards (Sigma). Each sample was analyzed at least for 3 times.

Results and Discussion

The application of the three resistance inducers two days prior to inoculation with the pathogen lead to a remarkable reduction in infection rate of barley with powdery mildew to a degree between 45 % and 55 % (Table 1). The resistance induction could be inhibited by Actinomycin D (data not shown). There are no direct effects of the inducers on the pathogen. In our experiments no treatment changes plant growth or show phytotoxicity.

Table 1
Infection densities of powdery mildew on induced resistant or control barley plants

Treatment	Concentration	Colonies/leaf	Infection density
Water (control)		119 a[1]	100 %
B 50	culture filtrate	54 b	45 %
Trigonelline	10 µmol/l	52 b	44 %
Isonicotinic acid methyl ester	10 µmol/l	65 b	55 %

[1] Values followed by the same letter do not differ significantly at $p < 0.05$, Tukey-test.

At the chromatin level the following results can be achieved: With the method of STEINMÜLLER and APEL (1986) high molecular weight DNA can be extracted from barley plants. There were no differences in the molecular sizes of the DNA isolated from the chromatin of induced resistant or control plants (Fig. 1).

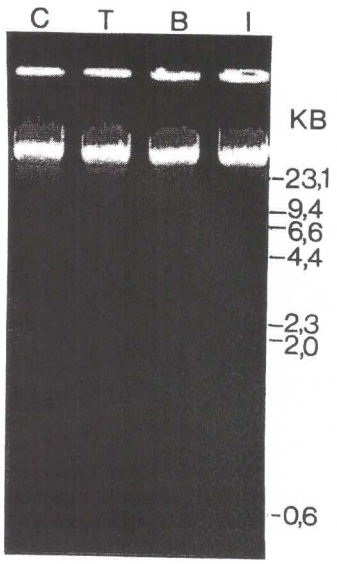

Fig. 1. Analysis of DNA isolated from chromatin of barley primary leaves 2 days after treatments. C: control (= water), T: Trigonelline, B: B 50, I: Isonicotinic acid methyl ester. Marker: Lambda DNA digested with HindIII

After hydrolization of the purified heigh molecular weight DNA we estimated the level of m^5-Cytosine with the HPLC, to find out, if changes in the chromatin structure occurred after resistance induction. A typical chromatogram of DNA bases is shown in Figure 2 with the buffer gradient we used, because of the better peak symmetry we could obtain in our HPLC equipment. Two days after application of the substances the level of genomic DNA methylation was

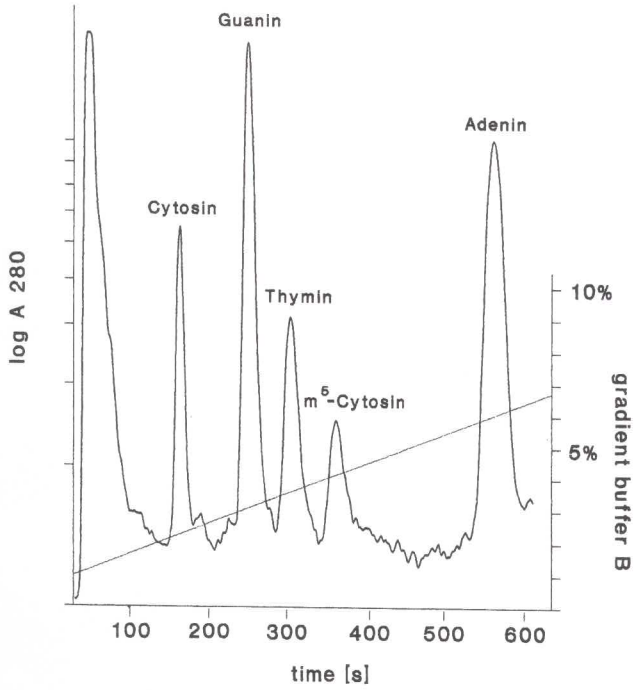

Fig. 2. A typical HPLC chromatogram of a DNA hydrolysate of barley primary leaf chromatin. A full-scale signal corresponds to 0.02 absorbance units at λ = 280 nm

reduced (Table 2). The decrease of cytosine methylation varied between 3.1 % and 4.8 % for the inducers. In comparison to the control (= 100 %) the reduction was 11.5 % for B 50, 16.7 % for Trigonelline and 17.8 % for Isonicotinic acid methyl ester respectively. The level of cytosine methylation in the control was 27 %. This is in the range for barley as compared to the literature (ADAMS and BURDON 1985, KLAAS and AMASINO 1989).

MESSEGEUR *et al.* (1991) reported a reduction of DNA methylation in immature tomato leaves compared to mature leaves of about 4.7 % and KLAAS *et al.* (1989) could reduce the methylation level of tobacco cell cultures with 5-azacytidine up to ⅓ of that found in untreated controls. In this connection the reduction of genomic methylation by resistance inducers as reported here seems to be possible and not the result of artefacts or disruption of cellular structures.

Table 2
Methylation levels of DNA isolated from induced resistant and control barley primary leaves 2 days after treatment

Treatment	%m^5 Cyt	Reduction of %m^5 Cyt	
		absolute [%]	relative [%]
Water (control)	27.0 a[1]	—	—
B 50	23.9 b	−3.1	−11.5
Trigonelline	22.5 b	−4.5	−16.7
Isonicotinic acid methyl ester	22.2 b	−4.8	−17.8

[1] Values followed by the same letter do not differ significantly at $p < 0.05$, Tukey-test after data transformation.

KLAAS *et al.* (1989) find the demethylation 2 days after treatment with methylation inhibitors. This result shows similarities with our findings, because the process of induction requires 2 days to establish the resistance in plants against pathogens (STEINER 1989) and we could also find a decrease in cytosine methylation after 2 days.

In our experiments the reduced level of genomic methylation always occurred with the induced resistance of the plants against obligate biotrophic fungi. This coincidence supported the hypotheses that the gene regulation is altered in some way after application of the inducers in barley. Certain similarities in their mode of action compared to DNA-methylation inhibitors as described in ADAMS and BURDON (1985) or KLAAS *et al.* (1989) are obvious, especially the decrease in DNA methylation. From this experiments we could not conclude which genes or parts of the genome are affected by the inducers and if these changes in the chromatin only occurred in resistance genes. Moreover we could not say if this demethylation is also found for single genes. These points have to be clarified in the next future.

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