Host Gene Expression at an Early Stage of Virus Resistance Induction

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Abstract

Suppression subtractive hybridization (SSH) was carried out to detect genes differentially expressed in plants expressing resistance to systemic infection with *Potato virus A* (PVA), genus *Potyvirus*. Differential screening has up to now revealed 19 putative differentially expressed genes. Nothern blot hybridization has confirmed the differential expression of seven genes. Three of them were only induced by the virus, but four genes were also wound-induced.

Keywords: PVA; *Solanum tuberosum*; suppression subtractive hybridization

INTRODUCTION

The aim of our study is to detect differentially expressed genes in potato (*Solanum tuberosum*) at an early stage of resistance induction upon infection with *Potato virus A* (PVA), a +ssRNA containing picorna-like potyvirus, by comparing susceptible plants to plants with hypersensitive resistance (HR) or non-hypersensitive resistance (non-HR), both of which prevent the phloem-dependent movement of PVA. The plant material used in this study is a diploid mapping population segregating for resistance to PVA. The population has earlier been inoculated with PVA and the plants grouped to phenotypic classes depending on their response to PVA (HÄMÄLÄINEN *et al*. 2000). HR is controlled by the dominant gene Na_{adg} and it blocks vascular transport of PVA. Cell death is induced and necrotic lesions develop on inoculated leaves. HR does not prevent virus replication; virus can be detected in leaf areas surrounding the necrotic lesion. Non-HR also blocks vascular transport of PVA, but no cell death and no symptoms can be detected. Non-HR does not prevent virus replication; PVA is detectable but only in inoculated leaves. Non-HR is controlled by the recessive gene *ra*. To isolate differentially expressed genes suppression subtractive hybridization (SSH) can be used. In this method sequences only present in the tester cDNA (i.e. cDNA from virus-resistant plants) can be detected by comparison to driver cDNA (i.e. cDNA from virus-susceptible plants). Sequences expressed exclusively, or to higher levels, in the tester are enriched and subsequently equalized through hybridization kinetics and PCR (DIATCHENKO *et al*. 1996).

MATERIALS AND METHODS

Progeny lines were pooled in three groups according to their phenotype; susceptible, HR or non-HR. Each group contained four progeny lines (two plants of each). The infectous cDNA clone of PVA-B11 was inoculated by shooting as described by RÄJAMÄKI and VALKONEN (1999). The sampling was done before inoculation and at 6, 12, 24 and 48 hours after inoculation. Leaves from plants in the same group were pooled during the sampling procedure. Leaves were ground in liquid nitrogen and about 0.5–1 g was used for RNA extraction. mRNA was purified and cDNA was synthesized and used for SSH. The SSH PCR products were cloned and 360 clones were screened to identify clones that might be differentially expressed. The identified clones were sequenced. Differential expression was confirmed by Nothern blot hybridization.

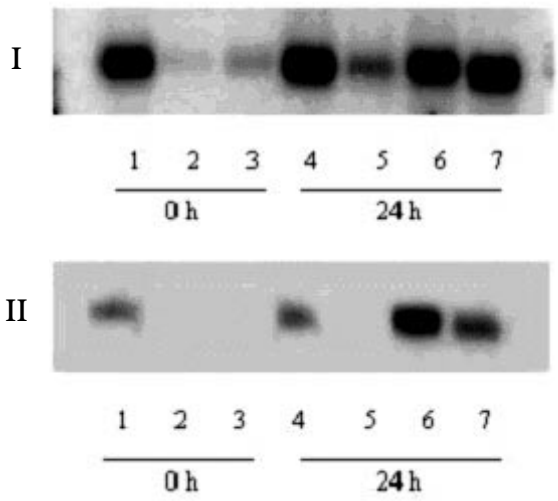

Figure 1. Number corresponds to following PVA-B11 inoculated samples: 1) HR, 2) S, 3) non-HR, 4) HR, 5) S, 6) non-HR and 7) mockinoculated non-HR. Nothern blot analysis showing. I) gene expressed differentially between non-HR and S, but also wound-induced and expressed before inoculation in HR plants. II) gene expressed more in virusinoculated plants than mockinoculated plants

RESULTS

Of 400 clones, 42 genes were differentially expressed in non-HR plants, according to screening. When these clones were sequenced 19 different genes were found. One gene was represented by 10 cDNAs another one as five cDNAs, and a third one as two copies. Most of these genes have previously been found to be induced in plants suffering from different kinds of stresses. Of these genes, the differential expression of seven genes has been confirmed by Nothern blot hybridization (as tested by 24 hours post virus inoculation). Three of them are induced to a higher degree in virus inoculated plants than in mockinoculated plants (Figure 1), whereas the other four genes are induced to the same extent in the virusinoculated and mockinoculated plants, but are not induced in virus-susceptible plants. In the Nothern blot analyses also RNA from HR plants before virus inoculation was included and six of the genes differentially expressed in non-HR plants are highly expressed already before virus inoculation in the HR-plants.

DISCUSSION

The results obtained so far suggest that common defence related genes are induced also in non-HR resistance where no symptoms develop at the sites of infection. To confirm that the differentially expressed genes really are connected to non-HR resistance, the expression of them in single plants rather than in the pool of plants tested up to now needs to be verified.

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