Temporal Expression of a *PGIP*-Gene in Strawberry Cultivars Induced by Wounding or by *Botrytis cinerea* Infection

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Abstract

The expression of a PGIP gene (polygalacturonase inhibitor protein) was monitored with semi-quantitative (SQ)-RT-PCR in green, white and red berries of the strawberry cultivar Korona upon infection with *Botrytis cinerea* and wounding. In addition, the PGIP expression in infected white berries was quantified in four additional cultivars. The constitutive expression of PGIP increased from green to red berries in Korona suggesting developmental regulation of the gene. Wounding and fungal infection caused a moderate or a high induction in the PGIP level, respectively. The maximum peak was observed 24 h after the treatments. In the comparative experiment with five cultivars, infection of white berries caused an induction in the PGIP level 24 h after inoculation in four out of five cultivars.

Keywords: strawberry; *Botrytis cinerea*; polygalacturonase inhibitor protein

INTRODUCTION

Fungal polygalacturonases (PGs) are among the first enzymes to be secreted upon infection and their action is a pre-requisite for other cell-wall degrading enzymes secreted later in the infection process. In response, plant cell walls contain glycoproteins which specifically and effectively recognize and inhibit PGs of fungal origin. To be able to defend against a range of PGs from pathogens, plants have evolved PGIPs with different recognition abilities. PGIPs from different plants have been shown to possess differential inhibitory activity towards PGs from various pathogens. The ability of PGIPs to inhibit fungal PGs can be seen as the sum of various PGIPs present in the host, each PGIP contributing to a range of inhibitory activities. PGIP has been induced in response to fungal infection, wounding, and application of elicitor (e.g. MACHINANDIARENA *et al*. 2001). Consequently, PGIP is included among the known classes of inducible defence-related cell wall proteins. PGIP is differentially regulated during development; in the fruits of some plant species, the constitutive expression increases with fruit maturity whereas in others it shows a decline.

By rotting the fruit, the fungus *Botrytis cinerea* is a major pathogen limiting strawberry production. Strawberry cultivars vary in susceptibility to *B. cinerea* (DAUGAARD 1999). This study presents data on the PGIP expression in selected strawberry cultivars after infection with *B. cinerea* or wounding.

MATERIALS AND METHODS

Disease controlled, flower-induced strawberry (*Fragaria* × *ananassa*) plants of cv. Korona, Senga sengana, Elsanta, Tenira and Polka were grown in pots in greenhouse at 16 h daylight and 18–20°C. The plants were completely randomized in three blocks. Sterile destilled water containing tween 20 was used to collect conidia of *B. cinerea* (strain No. 700, IPO-DLO, Wageningen) from potato dextrose agar plates. The number of conidia was adjusted to 10^5 per ml and 0.5 ml of conidial suspension was applied with a syringe into each inoculated berry. To secure infection, the plants were covered with a

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plastic bag for 24 hours after treatment. In the first experiment, green, white and red berries of cv. Korona were inoculated with *B. cinerea* or wounded and samples were taken 0–4 days after treatment. In the other experiment, white berries of the five cultivars were similarly inoculated with *B. cinerea* and sampled 24 h after inoculation.

RNA was extracted according to SCHULTZ *et al.* (1994). Two microgram of total RNA from each treatment was reverse transcribed using Superscript reverse transcriptase (Gibco BRL) and oligo $(dt)_{12-18}$ primers following the manufacturer's instructions. One microliter of diluted cDNA (1/20 in MQ) was used as a template for PCR. As a reference gene, a constitutively expressed DNA binding protein was employed (SCHAART *et al.* 2002). For PGIP, primers were designed from the recently cloned gene from cv. Elsanta (MEHLI & SCHAART – unpubl. data). A cycle number corresponding to the linear phase of the PCR reaction was identified as 30 cycles for both genes. The dbp-gene was used in the subsequent PCR to normalize each sample for PGIP amplification.

RESULTS AND DISCUSSION

The constitutive expression of the PGIP is at a low level in green and white berries (Figure 1), whereas relatively high levels were recorded in the red berries (data not shown). This suggests that in cv. Korona this gene is up-regulated during maturation. The now observed up-regulation of PGIP differs from that observed in tomato where the gene is down-regulated with fruit maturation (POWELL *et al.* 2000). In green berries, both wounding and infection induced PGIP expression and in both cases the maximum level was recorded 24 h after the treatment. Rapid induction of PGIP due to fungal infection and wounding has also been observed in other plants (e.g. MACHINANDIARENA *et al.* 2001; BÉZIER *et al.* 2002). Compared to wounding, the induction by infection was almost two-fold higher. In white berries the induction by infection was at the same level as in green berries, whereas wounding had no impact on the expression level. In red berries the constitutive expression of PGIP was up to eight-fold higher than in the green berries. Individual variation between mature red berries was considerably large and no clear induction or decline was observed in the expression level of PGIP in different treatments (data not shown).

The five strawberry cultivars included in the study display differential susceptibility towards *B. cinerea*. Tenira, Elsanta and Polka are the least susceptible, while Senga sengana is the most susceptible. Korona is regarded as being moderately susceptible (DAU-

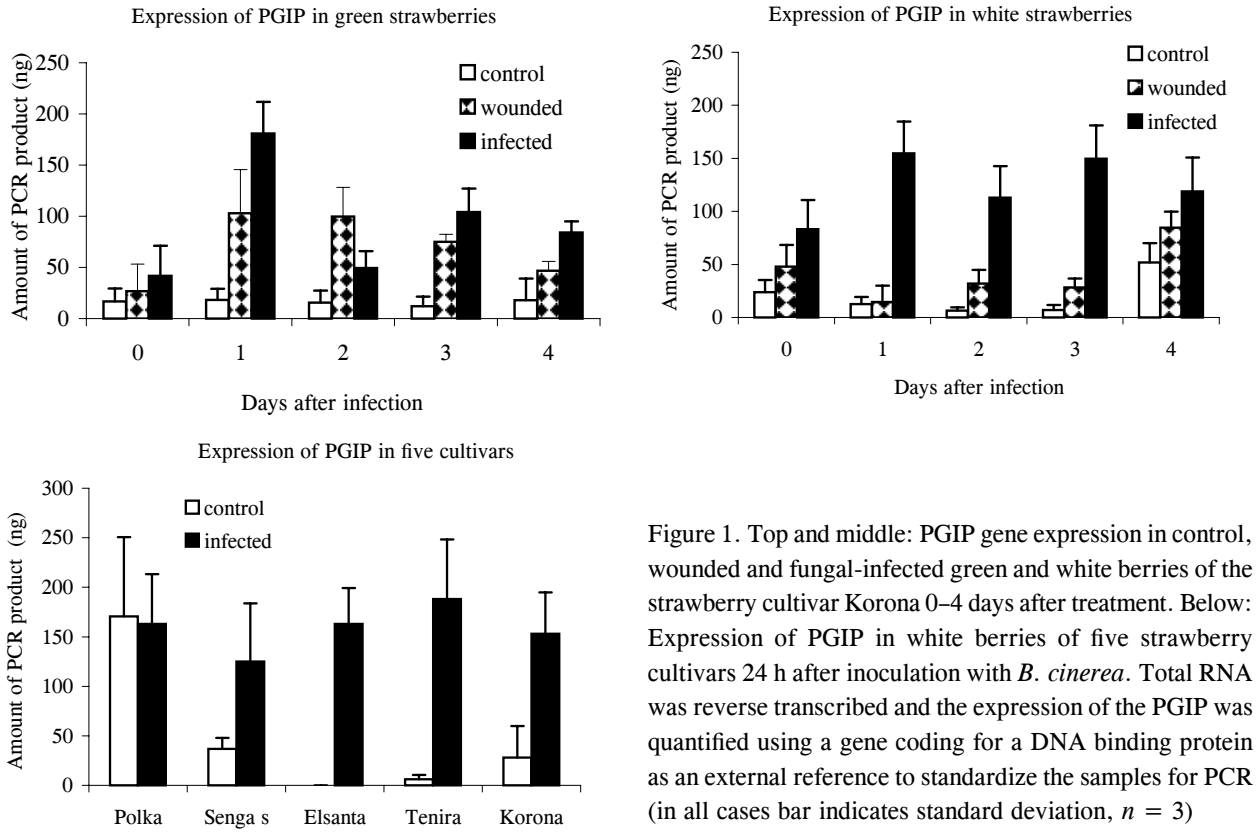

Figure 1. Top and middle: PGIP gene expression in control, wounded and fungal-infected green and white berries of the strawberry cultivar Korona 0–4 days after treatment. Below: Expression of PGIP in white berries of five strawberry cultivars 24 h after inoculation with *B. cinerea*. Total RNA was reverse transcribed and the expression of the PGIP was quantified using a gene coding for a DNA binding protein as an external reference to standardize the samples for PCR (in all cases bar indicates standard deviation, $n = 3$)

GAARD 1999). All cultivars except Polka showed low constitutive expression of PGIP (Figure 1). Infection induced PGIP at a higher level in Elsanta and Tenira than in the other cultivars. These results are consistent with the reported susceptibility of the cultivars. Further experiments are being carried out to verify the high constitutive expression in Polka.

The now used (SQ)-RT-PCR is tedious and problematic as it does not allow the monitoring of the target gene and the reference gene in the same PCR reaction. Real-time PCR is increasingly receiving attention as a way to quantify infection agents and host and pathogen transcripts. The option for multiplexing and the assay sensitivity make this technique attractive and further analyses on the topic of this paper are presently being carried out with this tool.

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