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BRIEF COMMUNICATION

# Ubiquitin messenger RNA accumulation in potato leaves as a response to the pathogenic fungus *Phytophthora infestans*

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## Abstract

The changes in ubiquitin mRNA level in intact plants of both susceptible (cv. Spunta) and partially resistant (cv. Pampeana-INTA) potato cultivars after inoculation with low doses ($10^3$ sporangia $cm^{-3}$) of *Phytophthora infestans* were studied after 72 h of treatment. Inoculation leads to 5-fold accumulation of potato ubiquitin transcripts in both cultivars. This result supports the connection between ubiquitin expression and defense reaction in plants.

*Additional key words*: resistant and susceptible cultivars, *Solanum tuberosum*.

Two types of potato resistance to *Phytophthora infestans* (*P.i.*) have been described: (*a*) specific resistance, which is controlled by simple dominant R-genes (Spielman *et al.* 1992) and functions only against certain races of the fungus and (*b*) general resistance, which is also known as field resistance or non-race specific resistance. The latter is a polygenically inherited character and functions against all races of the fungus (Umaerus 1970). Following the pathogen attack, a set of genes is activated in potato plants leading to the development of processes such as: *1*) production of callose-like material (Cuypers and Hahlbroock 1988); *2*) accumulation of PR proteins (Schroder *et al.* 1992); *3*) induction of the enzymes of the

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*Abbreviations*: *P.i.* - *Phytophthora infestans*; PR - pathogenesis related

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phenyl propanoid pathway and enzymes of biosynthetic pathway of lignin (Fritzemeier *et al.* 1987).

Ubiquitin is a highly conserved protein encoded by multigene family. Some members of the family are constitutively expressed, while others are regulated by development or environmental conditions (Monia *et al.* 1990). In plant systems, activation of ubiquitin gene expression has been observed in protoplasts of *Nicotiana sylvestris* following virus attack (Geschnik *et al.* 1992). On the other hand, Pieterse *et al.* (1991) infecting isolated potato leaves with $10^5$ sporangia $cm^{-3}$ of *P.i.*, found induction of *P.i.* ubiquitin gene while no induction of potato ubiquitin genes.

In this report, we show evidences on the accumulation of ubiquitin messenger RNA in *P.i.*-infected potato leaves in a whole plant infection system.

Plants of potato (*Solanum tuberosum* L.) cvs. Spunta and Pampeana-INTA were chosen because they show a range of non-race specific resistance to late blight in the field (low and high resistance, respectively - M. Huarte, personal communication). They were grown in a mixture of sterile vermiculite and soil (1:3) in pots (diameter 20 cm) at 20 °C in a chamber with 14 h photoperiod and an irradiance of 250 µmol(PAR) $m^{-2}$ $s^{-1}$ during 1 month. Then, plants were sprayed with $10^3$ sporangia $cm^{-3}$ of *P.i.* at the beginning of the darkness period and incubated at 18 °C and 100 % RH in a chamber with a transparent lid under the initial conditions of irradiance. As a control, plants were sprayed with water in the same way. Under these conditions of inoculation, the symptoms of the late blight disease were not visible until 72 h. During this period leaves were collected for RNA extraction and further analysis.

Isolate "INTA" of *P.i.* (A2-mating type, race 1, 4, 7, 8, 10, 11) was obtained from a naturally infected potato plant and kindly supplied by Ing. Agr. Marcela van Damme, INTA, Balcarce, Argentina. *P.i.* was maintained on V8-agar medium containing 50 µg $cm^{-3}$ ampicillin (*Serva*), 100 µg $cm^{-3}$ penicillin (*Richet*) and 30 µg $cm^{-3}$ rifamycin. *P.i.* was also grown on potato tuber slices for RNA extraction and to prepare sporangia (Pieterse *et al.* 1994) for infection experiments. Tuber were thoroughly washed with 2 % Na hypochloride, rinsed with 70 % ethanol and flamed. Tuber slices were inoculated with *P.i.* and put at 18 °C in the dark with 100 % RH. Mycellia were harvested from potato tuber at 5 d post-inoculation into sterile water (4 °C) and sporangia concentration was adjusted to $10^5$ sporangia $cm^{-3}$.

Total RNA from both potato leaves and mycellium of *P.i.* grown on potato tubers were prepared using the guanidine hydrochloride method described by Logeman *et al.* (1987). The RNA was electrophoresed in 1 % agarose denaturating gels containing 7 % formaldehyde.

The cDNA clone encoding ubiquitin (Binet *et al.* 1991) and rDNA clone encoding 18S and 28S of rRNA were obtained from sunflower and were used as probes in Northern blots analysis. Probes were labelled using the "random priming" kit (*Dupont*, USA) following the manufacturers instructions. For Northern blot analysis, 20 µg of total RNA were denatured, electrophoresed and its integrity checked by ethidium bromide staining. The RNA was transferred to Hybond-N membrane (*Amersham*) and hybridized at 42 °C in 50 % formamide, 5 × SSPE, 0.5 %

Denhardt's solution and 0.5 % SDS. The blot was washed in 0.1 % SSPE, 0.1 % SDS at 42 °C and exposed to Kodak X-Omat film.

Considering that isolated potato leaves were used in the experiments performed by Pieterse *et al.* (1991) and that those leaves were under the stress of mechanical injury, it was of interest to investigate the level of ubiquitin transcripts in leaves attached to the plants upon inoculation with low doses of *P.i.* sporangia ($10^3$ $cm^{-3}$). Fig. 1 shows total RNA extracted from intact leaves at different times after

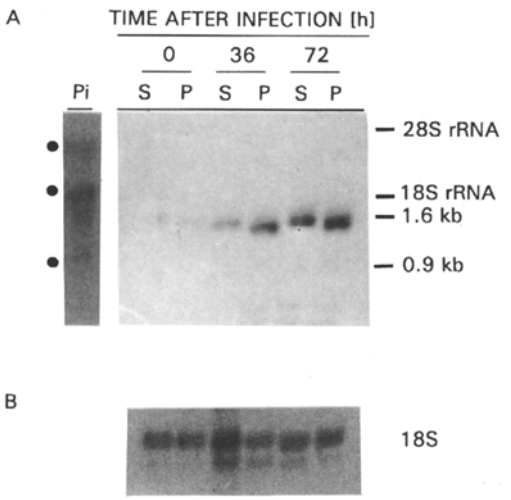


Fig. 1. Northern blot analysis of the potato ubiquitin mRNA. Inoculated plants at the indicated time were used for RNA extraction and blot analysis. Blots were hybridized with the ubiquitin cDNA (*A*) and with rDNA probes (*B*). Pi: RNA from *Phytophthora infestans* grown on potato tuber. S: potato cv. Spunta; P: potato cv. Pampeana-INTA. Dots denote position of bands from *Phytophthora infestans* RNA sample.

inoculation and probed with an ubiquitin cDNA clone. The ubiquitin probe hybridized predominantly to an unique size of mRNA of 1.6 kb. This transcript might correspond to 5 or 6 ubiquitin coding units as reported in potato tubers (Garbarino *et al.* 1992). After 72 h of treatment, ubiquitin messenger RNA concentration in inoculated leaves was 5-fold higher than in plants processed at the time of inoculation (0 h) (Fig. 1). Control plants sprayed with water at different times, did not show variations in ubiquitin mRNA (data not shown). The increase in the level of this transcript was visible 36 h after inoculation and was similar in both potato cultivars at 72 h, suggesting that the ubiquitin induction could be part of the general response occurring in plants after the challenge of pathogen. Moreover, it was found that tobacco plants perturbed in the ubiquitin system leads to changes in the response of both susceptible and resistant plants to viral infection (Becker *et al.* 1993). In addition, preliminary results in our laboratory indicate that ubiquitin expression decreased in intact potato plants sprayed with $10^5$ sporangia $cm^{-3}$ of *P.i.* Under these conditions, symptoms of disease were clearly visible 48 h after inoculation of plants (not shown).

No signal for fungal ubiquitin transcript was detected in RNA extraction from infected potato leaves (Fig. 1). It was consistent with the absence of symptoms of the late blight disease at 72 h after infection and no detection of fungal biomass. This may be probably due, to the low concentration of sporangia ($10^3$ sporangia $cm^{-3}$) used in our inoculation experiments which led to a very low development of necrotic spots on plant tissues (not shown). Since a correlation exists between concentration of sporangia used for infection and the fungal biomass developed on potato foliage, a lower biomass of fungi should be detected in our experimental conditions ($10^3$ sporangia $cm^{-3}$) in contrast to $10^5$ sporangia $cm^{-3}$ used for infection in Pieterse (1991) experiments. Fig. 1 also shows that in Northern blot analysis, the sunflower ubiquitin probe detected three transcripts in RNA preparations from *P.i.*, corresponding the 0.9 kb transcript to the previously reported (Pieterse *et al.* 1991). However, none of them corresponded in size, to the potato ubiquitin transcript, being consistent with the absence of fungal biomass.

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