TRANSGENIC POTATO EXPRESSING TWO ANTIFUNGAL PROTEINS

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Introduction

Transgenic crops with enhanced resistance against fungal diseases have been achieved through the constitutive expression of chitinase (1), thaumatin-like protein (2) and β-1,3-glucanase (3). However, considering that antifungal proteins have often synergistic activities and that pathogens can overcome the resistance conferred by individually transferred traits, combinatorial expression of antifungal proteins in transgenic crops is regarded as a key for effective and durable field resistance. Recently, tobacco (3) and tomato (4) plants constitutively expressing tobacco class I chitinases and class I β-1,3-glucanases exhibited substantially enhanced resistance against fungal attack.

We present preliminary data on field performance of transgenic potato plants co-expressing a tobacco class I β-1,3-glucanase and the thaumatin-like protein AP24.

Materials and Methods

The tobacco coding genes for a class I β-1,3-glucanase and AP24 were independently placed under the control of the CaMV 35S promoter with a synthetic 65 bp non-coding 5' leader sequence of TMV and the nos transcription terminator. A double construct carrying the chimeric β-1,3-glucanase and ap24 genes in tandem was used for constitutive co-expression of the two proteins in potato. The expression cassettes were separetely inserted in the binary vector pDE1001 and transferred into *Agrobacterium tumefaciens* AT2260. Transgenic tobacco and potato plants were generated by leaf disc transformation. Transformants were selected on MS medium containing kanamycin. PCR was used to detect the presence of the glucanase and ap24 transgene sequences. ELISA and western blotting were used to determine transgene expression. *In vitro* antifungal assays were carried out by using tobacco leaf extracts.

Screening for enhanced antifungal resistance in transgenic potato was carried out under field conditions. One hundred kanamycin-resistant clones were micropropagated, transferred to soil and grown for ten days under greenhouse conditions. Then, ten plants of each clone and non-transformed control were planted in field and subjected to natural infection. The degree of fungal infection was established in a scale from 0 to 4 according to the presence of disease symptoms in plants.

Results and Discussion

The transgene-encoded β-1,3-glucanase and AP24 were expressed in tobacco at levels between 0.1 and 0.3 % of total proteins. The engineered proteins were correctly processed as indicated by western blotting, suggesting their targeting to the cell vacuole. The constitutive expression of AP24 resulted in an increased antifungal activity of the tobacco leaf extracts against *Rhizoctonia solani* and *Fusarium solani*. Transgene expression in tobacco was used to confirm the functionality of the chimeric glucanase and ap24 constructs.

A double construct for constitutive co-expression of β-1,3-glucanase and AP24 was used to transform the potato commercial cultivar Désirée, One hundred kanamycin-resistant clones were screened for antifungal resistance under field conditions. After sixty days, most of the plants were severely attacked by *Alternaria solani* and *Phytophthora infestans* although showing different levels of disease symptoms. Three clones were distinguished for a low degree of fungal infection. Experiments to correlate levels of transgenes expression and antifungal resistance are in progress.

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