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# Transgenic strawberry plants expressing a thaumatin II gene demonstrate enhanced resistance to *Botrytis cinerea*

K.A. Schestibratov *, S.V. Dolgov

*Artificial Climate Station "Biotron", Branch of Shemyakin and Ovchinnikov, Institute of Bioorganic Chemistry RAS, Science Avenue 6, 142290 Pushchino, Moscow Region, Russia*

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**Abstract**

A thaumatin II cDNA (*thau II*) driven by the CaMV 35S promoter was introduced into strawberry (*Fragaria* × *ananassa*) via *Agrobacterium*-mediated transformation. Following transformation, 19 regenerated plant lines were obtained, of which, 14 were found to possess *thau II* and neomycin phosphotransferase (*npt II*) genes, while five plants possessed only the *npt II* gene. In 11 transformants, protein bands that reacted with a thaumatin II antibody were observed, while 3 plants had no detectable thaumatin II. Three transgenic lines accumulating the highest levels of thaumatin II protein and three control plants with *npt II* and *uidA* genes or only the *npt II* gene and non-transgenic plants cv. Firework were tested for resistance against gray mold (*Botrytis cinerea*) by infection with a conidial suspension. All transgenic lines tested expressing the thaumatin II protein showed a significantly higher level of resistance compared to the control plants, as measured by necrotic lesions and sporulation of the pathogen.


*Keywords:* Strawberry; Transformation; *Agrobacterium tumefaciens*; *Botrytis cinerea*; Disease resistance

*Abbreviations:* BA, benzyl adenine; CaMV, cauliflower mosaic virus; IBA, 3-indolebutyric acid; MS, Murashige and Skoog (1962) medium; *npt II*, neomycin phosphotransferase II gene; Pnos, promoter of T-DNA nopaline synthase gene; PR, pathogenesis-related; TDZ, thidiazuron

* Corresponding author. Tel.: +7 095 925 23 42; fax: +7 0967 33 05 27.
*E-mail address:* schestibratov@rambler.ru (K.A. Schestibratov).

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## 1. Introduction

Strawberry is an important small fruit crop grown worldwide, and its production is increasing steadily. Many cultivars of strawberry have already been bred, thereby allowing this crop to be cultivated under various conditions. However, there are still many factors which limit plant yield significantly, one of which is susceptibility to a variety of phytopathogenic fungi, bacteria and viruses. Gray mold or *Botrytis* fruit rot, caused by *Botrytis cinerea*, is one of the most destructive diseases of strawberry, causing substantial yield loss before and after harvest. Cultivars can be susceptible, moderately susceptible and minimally susceptible, but no cultivar is immune (Jarvis and Borecka, 1968; Simpson, 1991; Sutton, 1998). Thus, gray mold resistance in strawberry plants is one of the most important traits to have been pursued in breeding programs over several decades. Due to the unavailability of breeding material with resistance against gray mold, to date disease resistance breeding has not progressed well. In this case, a transformation technique can be a useful method to produce novel breeding materials because this can make use of isolated genes from a great number of species.

The development of gene transfer techniques for crop plants has facilitated testing constitutive overexpression of proteins involved in the plant defense system. Plant resistance to pathogens involves the accumulation in the plant cell of proteins active in defense mechanisms such as PR-proteins. The accumulation of these proteins has been shown to be correlated with the development of systemic acquired resistance in plants (Van Loon, 1997; Kasprzewska, 2003). Typically, these anti-fungal proteins are expressed constitutively at low levels in cells and accumulate in response to fungal attack or in response to other inducers of acquired resistance. However, these mechanisms are often too weak or appear too late to be effective in protecting the plant. This strategy involves the constitutive overexpression of defense proteins that have inhibitory activity against the hyphae and/or germinating spores of the pathogen. Among these are PR proteins including glucanases (Yoshikawa et al., 1993), chitinases (Broglie et al., 1991) and thaumatin-like proteins (Liu et al., 1994). This seems to be a promising strategy also for the development of gray mold resistant strawberry.

Several proteins belonging to the PR-5 group have been used successfully to enhance plant resistance to fungal pathogens (review: Punja, 2001). For example, Zhu et al. (1996) demonstrated that transgenic potato plants constitutively expressing high levels of an osmotin-like protein, a member of the PR-5 group, showed increased resistance to the late blight fungus, *Phytophthom infestans*. Also transgenic rice plants with constitutive expression of rice thaumatin-like protein (TLP) had enhanced resistance to the pathogen *Rhizoctonia solani* (Datta et al., 1999). Enhanced resistance of transgenic wheat plants to *Fusarium graminearum* due to the integration and expression of rice TLP was also demonstrated (Chen et al., 1999). Recently, Szwacka et al. (2002) produced transgenic cucumber plants with constitutive expression of a thaumatin II gene from *Thaumatococcus daniellii* (Edens et al., 1982), under control of the cauliflower mosaic virus (CaMV) 35S promoter. The transgenic plants and their progeny expressing thaumatin II protein showed enhanced resistance against the pathogenic fungus *Pseudoperonospora cubensis*. However, some of the $T_2$ progeny plants expressing thaumatin II protein did not exhibit increased resistance to *P. cubensis*.