Crown Rust Resistance in Transgenic Italian Ryegrass (*L. Multiflorum*) Expressing a Rice Chitinase Gene and Crosses with Cytoplasmic Male Sterile Hybrid Ryegrass

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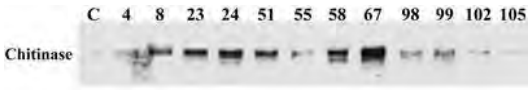
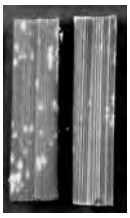
Keywords: disease resistance, *Puccinia coronata*, particle bombardment, cytoplasmic male sterility

Introduction. Italian ryegrass (*Lolium multiflorum* Lam.) is one of the most important forage grasses in the temperate region. In ryegrasses, crown rust (*Puccinia coronata*) is the most serious foliar fungal disease and brings about a reduction of herbage yield and loss of palatability to grass-eating domestic animals. In this study, we tried to increase tolerance to the pathogen by introducing a rice chitinase gene using particle bombardment.

Materials and methods Genetic co-transformation of Italian ryegrass (W3 genotype from cultivar Waseaoba) was performed with the HPT gene (pAcH1) and a rice chitinase gene (pAcC-RCC2) (Takahashi *et al*. in press). Hygromycin resistant transgenic plants were checked by PCR and Southern hybridisation was used to determine whether they possessed the rice chitinase gene. Northern analysis was also carried out. Transgenic plants carrying the rice chitinase gene were evaluated for their tolerance to crown rust by the inoculation of detached leaves with spores. Flowering transgenic plants carrying rice chitinase genes were crossed to a cytoplasmic male-sterile hybrid ryegrass in order to produce pollen-less transgenic disease resistant breeding material.

Results We checked the integration of foreign genes by PCR analysis in the 72 regenerants that formed green shoots. Of these, 58 plants were positive for the *HPT* gene, and 39 bore the *RCC2* gene. The different Southern hybridization patterns observed among the transgenic plants indicated that they resulted from independent transformation events. A stronger Northern hybridization band was observed in most transgenic plants compared to non-transgenic plants, but we could not distinguish the *RCC2* gene transcript from that of the endogenous chitinase gene (Figure1). In both non-transgenic and *RCC2* gene expressing transgenic plants, macroscopic regions of chlorosis were observed about 7 days after inoculation with *P. coronata* spores. However we were able to distinguish differences in the severity of disease symptoms between them by 10 days after inoculation. The lowest number of lesions was observed in plant 67 (Figure 2). However, the disease continued to gradually invade the inoculated leaves of the transgenic plants as the culture period was prolonged (>10 days), and the disease symptoms of the transgenic plants developed to the same degree as those of the non-transgenic plants about 13 days after inoculation. Transgenic plants expressing the *RCC2* gene were fertile and were crossed to a cytoplasmic male-sterile hybrid ryegrass (F_1 of Italian ryegrass carrying male-sterile cytoplasm x perennial ryegrass), whose progenies show stable male sterility. Inheritance of the *RCC2* gene in the progenies was confirmed by PCR. Tolerance to rust and male sterility is being checked further in the progenies.





Conclusions Transgenic Italian ryegrass plants carrying the rice chitinase (*Cht-2*; *RCC2*) gene were obtained by particle bombardment and its expression was confirmed by molecular analysis. Bioassay of detached leaves indicated increased resistance to crown rust (*Puccinia coronata*) in the transgenic plants. Progenies between the transgenic plants and cytoplasmic male-sterile hybrid ryegrass were obtained.

References

Takahashi, W., M. Fujimori, Y. Miura, T. Komatsu, Y. Nishizawa, T. Hibi and T. Takamizo (2005) Increased resistance to crown rust disease in transgenic Italian ryegrass (*Lolium multiflorum* Lam.) expressing the rice chitinase gene. Plant Cell Reports, *In press*.