Development and field evaluation of transgenic ryegrass (*Lolium* spp.) with down-regulation of main pollen allergens

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Introduction Ryegrass (*Lolium* spp.) pollen is a widespread source of airborne allergens and is a major cause of hayfever and seasonal allergic asthma, which affect approximately 25% of the population in cool temperate climates. The main allergens of ryegrass pollen are the proteins Lol p 1 and Lol p 2. These proteins belong to two major classes of grass pollen allergens to which over 90% of pollen-allergic patients are sensitive. The functional role *in planta* of these pollen allergen proteins remains largely unknown. The generation, analysis and field evaluation of transgenic plants with reduced levels of the main ryegrass pollen allergens, Lol p 1 and Lol p 2 in the most important worldwide cultivated ryegrass species, perennial ryegrass (*L. perenne* L.) and Italian ryegrass (*L. multiflorum* Lam.) are described.

Materials and methods *Lol p 1* and *Lol p 2* cDNA and genomic clones were isolated. Transformation vectors were generated with *Lol p 1* and *Lol p 2* cDNA sequences in antisense orientation under the control of maize and ryegrass pollen-specific promoters.

Results and conclusions Embryogenic suspension cells of perennial and Italian ryegrass were subjected to biolistic transformation with *Lol p 1* and *Lol p 2* antisense vectors and transgenic plants were recovered. The transgenic nature of the perennial and Italian ryegrass plants was confirmed by Southern hybridisation analysis. Transgenic antisense *Lol p 1* and *Lol p 2* ryegrass plants showed a reduction in the levels of the respective pollen allergens assessed with antibodies raised against the recombinant allergenic proteins. Hypo-allergenicity of Lol p 1 down-regulated pollen was confirmed by immunoblots using IgE sera from Lol p 1 sensitised patients. Transgenic antisense *Lol p 1* ryegrass plants showed normal reproductive development and pollen viability. Selected antisense *Lol p 1* transformation events were evaluated in a small-scale field release carried out in Ardmore, Oklahoma, USA in 2004. Mitotic and meiotic stability of transgene integration and expression, as well as general morphology of the field-grown transgenic ryegrass plants were assessed. A more comprehensive assessment of pollen and gene flow of these transgenic ryegrass plants will be evaluated in a planned large-scale field release in USA in 2005. This will complement detailed gene and pollen flow studies undertaken in Hamilton, Australia using non-transgenic novel perennial ryegrass genotypes.

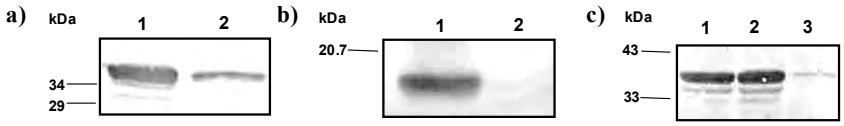

Figure 1 Western blot analysis of pollen protein extracts: a) using anti-Lol p 1 antibodies; untransformed control ryegrass plant (1) and Lol p1 down-regulated transgenic ryegrass plant; b) using anti-Lol p 2 antibodies; untransformed control ryegrass plant (1) and Lol p2 down-regulated transgenic ryegrass plant; c) using IgE antibodies from serum of grass pollen allergic patient; perennial ryegrass control plant (1), Italian ryegrass control plant (2) and Lol p1 down-regulated transgenic ryegrass plant (3)

Reference

Petrovska, N., Wu, X., Donato, R., Wang, Z.Y., Ong, E.K., Jones, E., Forster, J., Emmerling, M., Sidoli, A., O'Hehir, R. and Spangenberg, G. (2004) Transgenic ryegrasses (*Lolium* spp.) with down-regulation of main pollen allergens. *Molecular Breeding*, 14, 489-501.