



Development and Field Evaluation of Transgenic Ryegrass (*Lolium* Spp.) with Down-Regulation of Main Pollen Allergens

N. Petrovska

Molecular Plant Breeding Cooperative Research Centre, Australia

A. Mouradov

La Trobe University, Australia

Z. Y. Wang

The Samuel Roberts Noble Foundation

K. F. Smith

Hamilton Centre, Australia

G. C. Spangenberg

La Trobe University, Australia

Follow this and additional works at: <https://uknowledge.uky.edu/igc>



Part of the [Agricultural Science Commons](#), [Agronomy and Crop Sciences Commons](#), [Plant Biology Commons](#), [Plant Pathology Commons](#), [Soil Science Commons](#), and the [Weed Science Commons](#)

This document is available at <https://uknowledge.uky.edu/igc/20/satellitesymposium5/103>

The XX International Grassland Congress took place in Ireland and the UK in June-July 2005.

The main congress took place in Dublin from 26 June to 1 July and was followed by post congress satellite workshops in Aberystwyth, Belfast, Cork, Glasgow and Oxford. The meeting was hosted by the Irish Grassland Association and the British Grassland Society.

Proceedings Editor: D. A. McGilloway

Publisher: Wageningen Academic Publishers, The Netherlands

© Wageningen Academic Publishers, The Netherlands, 2005

The copyright holder has granted the permission for posting the proceedings here.

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

N. Petrovska^{1,2}, A. Mouradov^{1,2}, Z.Y. Wang^{1,3}, K.F. Smith^{2,4} and G.C. Spangenberg^{1,2}

¹Primary Industries Research Victoria, Plant Biotechnology Centre, La Trobe University, Bundoora, Victoria 3086, Australia ²Molecular Plant Breeding Cooperative Research Centre, Australia ³The Samuel Roberts Noble Foundation, Ardmore, Oklahoma, USA ⁴Primary Industries Research Victoria, Hamilton Centre, Hamilton, Victoria 3300, Australia Email: natasha.petrovska@dpi.vic.gov.au

Keywords: ryegrass, pollen allergens, transgenic plants, hypo-allergenic pollen, field evaluation

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.

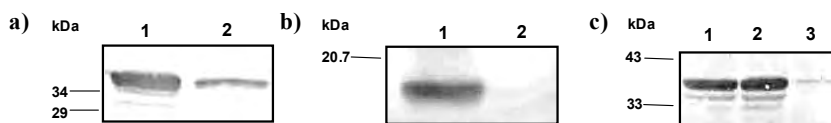


Figure 1 Western blot analysis of pollen protein extracts: a) using anti-Lol p 1 antibodies; untransformed control ryegrass plant (1) and Lol p 1 down-regulated transgenic ryegrass plant; b) using anti-Lol p 2 antibodies; untransformed control ryegrass plant (1) and Lol p 2 down-regulated transgenic 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 p 1 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.