LXR ™ white clover: development of transgenic white clover (*Trifolium repens*) with delayed leaf senescence

Y.H. Lin[1], J. Chalmers[1], E. Ludlow[1], C. Pallaghy[2], G. Schrauf[3], Pablo Rush[3], A.M. García[4], A. Mouradov[1] and G.C. Spangenberg[1,5]

[1]Primary Industries Research Victoria, Plant Biotechnology Centre, La Trobe University, Bundoora, Victoria 3086, Australia [2]Department of Botany, La Trobe University, Bundoora, Victoria 3086, Australia [3] Universidad of Buenos Aires, Catedra de Genetica, Facultad de Agronomia, Buenos Aires, Argentina [4]CIGEN-CONICET [5]Phytogene Pty. Ltd., Plant Biotechnology Centre, La Trobe University, Bundoora, Victoria 3086, Australia
E-mail: german.spangenberg@dpi.vic.gov.au

Keywords: white clover, senescence, transgenic plants, cytokinins, isopentenyl transferase

Introduction Leaf senescence is a type of programmed cell death characterized by loss of chlorophyll, lipids, protein, and RNA. Cytokinins are a class of plant hormones that play roles in many aspects of plant growth and development, including leaf senescence, apical dominance, the formation and activity of shoot meristems, nutrient mobilization, seed germination, and pathogen responses. They also appear to mediate a number of light-regulated processes, such as de-etiolation and chloroplast differentiation. It is known that the concentrations of endogenous cytokinins decline in plant tissues as senescence progresses. This observation provides the opportunity to manipulate the senescence program in transgenic plants to enhance biomass and seed production, through the regulated expression of cytokinin biosynthesis genes.

Materials and methods Transgenic white clover plants carrying a chimeric isopentenyl transferase (IPT) gene (*ipt*) from *Agrobacterium tumefacien*s under control of the *Arabidopsis thaliana atmyb32* promoter (Figure 1a) and a chimeric neomycin phosphotransferase gene (*npt2*) as selectable marker were generated. The enzyme IPT catalyzes the rate-limiting step for *de novo* cytokinin biosynthesis i.e. the addition of isopentenyl pyrophosphate to the N6 of 5'-AMP to form isopentenyl AMP.

Results and conclusions Following a PCR screening using *npt2* and *ipt* primers (Figure 1b), the transgenic nature of the white clover plants was confirmed by Southern hybridisation analysis revealing the integration of 1 – 10 T-DNAs in the genome of independent *atmyb32::ipt* white clover plants (Figure 1c). Expression of the *atmyb32::ipt* gene in transgenic white clover plants was confirmed by RT-PCR. Selected *atmyb32::ipt* white clover plants showed a marked delay in leaf senescence but otherwise developed normally. The delay in senescence was revealed by an increase in chlorophyll content in *atmyb32::ipt* leaves relative to leaves of untransformed and *atmyb32::gusA* control white clover plants. Delayed senescence was also observed in detached leaves. Detached leaves from selected *atmyb32::ipt* white clover plants (LXR™ white clover) showed no visible signs of senescence over 20 days post-detachment. Assessment under containment conditions of growth characteristics of LXR™ white clover plants revealed significant ($p<0.05$) increases in number of leaves, stolon length, total leaf area, cumulative and relative leaf area appearance rates compared with untransformed negative control plants. The first small-scale field release of selected LXR™ white clover transformation events has been established in Junin, Argentina in 2004.

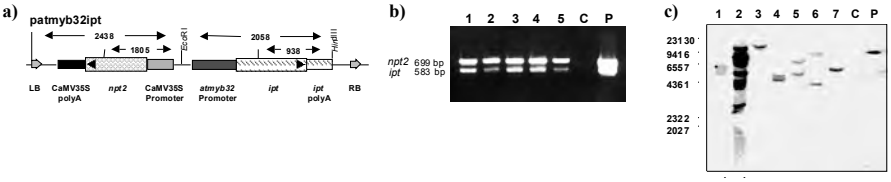

Figure 1 Molecular analysis of *atmyb32::ipt* white clover plants. a) Binary vector carrying chimeric *atmyb32::ipt* gene for the production of LXR™ white clover plants; b) PCR screening of LXR™ white clover plants using *npt2* and *ipt* specific primers; and c) Southern hybridisation analysis of LXR™ white clover plants using *ipt* hybridisation probe