Discovery, isolation and characterisation of promoters in white clover (*Trifolium repens*)

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Keywords: white clover, bacterial artificial chromosomes (BACs), BAC library, promoters, reporter genes

Introduction The availability of a suite of promoters with a range of spatial, temporal and inducible expression patterns is of significant importance to control targeted expression of genes for molecular breeding in forage species. A range of resources and tools have been developed for promoter isolation and characterisation in white clover (*Trifolium repens* L.), including a comprehensive BAC library and a 15K unigene microarray.

Materials and methods Discovery, isolation and characterisation of heterologous and endogenous promoters was undertaken in white clover. Expression patterns of chimeric *gusA* reporter genes encoding bacterial β-glucuronidase (GUS) with four differentially regulated promoters from *Arabidopsis thaliana* (*atmyb32*, *adh*, *xero2* and *SAG12*) were assessed in transgenic white clover plants generated by *Agrobacterium*-mediated transformation.

Results and conclusions Molecular analysis of independent transformants confirmed the stable integration of T-DNAs containing the various promoter-*gusA* reporter genes. Histochemical staining of plant tissues and organs revealed that the *atmyb32* promoter directed *gusA* expression in leaf and root vascular tissue including lateral roots and nodules with low levels of expression in reproductive organs. Wound-response of the a*tmyb32* promoter in white clover leaves and stolons was also shown. The *adh* promoter showed anaerobic stress and dehydration stress response. The *xero2* promoter directed strong expression in roots, leaf vascular tissue, inflorescences, anther filaments and pollen grains, while the *A. thaliana SAG12* promoter resulted in senescence-associated *gusA* expression in white clover leaves. A white clover BAC library consisting of 50,302 BAC clones with 101 kb average insert size, corresponding to 6.3 genome equivalents and 99% genome coverage was established. Root-prevalent promoters were isolated from white clover following screening of the BAC library. White clover BAC clones hybridising to phosphate transporter (*TrPT1*) and iron transporter (*TrRit1*) sequences were identified (Fig 1), and corresponding 5' regulatory sequences were isolated. Transgenic white clover plants expressing a chimeric *TrPT*::*gfp* gene encoding green fluorescence protein (GFP) fusion were produced. They revealed fluorescence in root tissues, mainly in root-tips and root nodules. This research provides a toolbox of promoters with a range of specificities for targeted gene expression as part of a molecular breeding approach in white clover deploying exclusively white clover genes and promoters for transgenic product development.

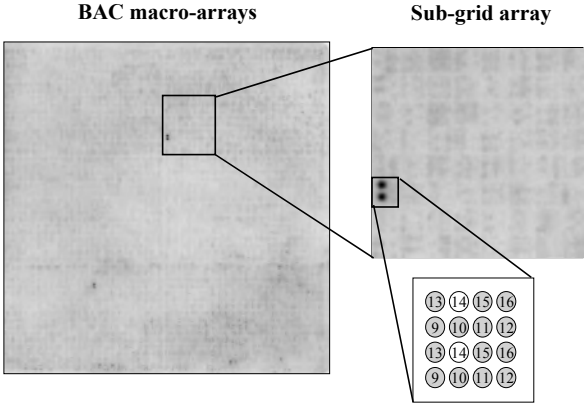

Figure 1 White clover BAC macro-array membrane after hybridisation with a *TrPT1* cDNA ^{32}P-labelled probe revealing *TrPT1*-hybridising genomic clones for *TrPT1* promoter isolation.