Minimising Bloat Through Development of White Clover (*T. Repens*) with High Levels of Condensed Tannins

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Introduction White clover constitutes a low percentage of the overall sward content in Irish pastureland despite EU directives limiting the use of nitrogenous fertilizers. This is mainly due to the tendency of large amounts of white clover to cause bloat. Bloat is a potentially fatal build up of proteinaceous foam in the guts of ruminants. Some lesser cultivated legumes such as *Lotus* species contain condensed tannins (CT) that decrease the incidence of bloated animals. The project's objective is to reduce the risk of bloat by generating white clover cultivars with high CT content. We are investigating whether expression of the *ANTHOCYANIN REDUCTASE* gene (*BAN*) in transgenic white clover and *Medicago truncatula* (model) plants leads to increased CT levels (Xie *et al*., 2003).

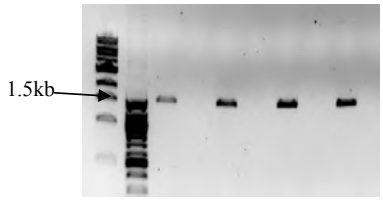
Materials and methods The *BAN* gene was isolated via PCR from *Arabidopsis thaliana* genomic DNA and is being introduced into pCAMBIA2300, under the expression control of the constitutive *UBIQUITIN3* promoter (ATUBP3), to form the plasmid vector pMTOD001. A number of white clover cultivars from the Oak Park germplasm collection were tested for their ability to regenerate into whole plants from cotyledons. The cotyledons were excised from imbibed seeds and transferred to shoot inducing media. Regenerated shoots were transferred to root inducing media to promote root formation (Ding *et al.,* 2003).

Results Optimal amplification of the *BAN* gene (1.5kb) was achieved at a $MgCl_2$ concentration of 4mM (Fig1). The regenerating results of the white clover cultivars from Oak Park's germplasm were found to vary from 0% to 47.5%. The best regenerating cultivars have been identified as Tara and Susi (Table 1). This choice was based on plants that had regenerated both roots and shoots.

Table 1 The regenerating efficiencies of a selection of legume cultivars

Species	Cultivar	Percentage regeneration
T. repens	Tara	47.50%
T. repens	Susi	37.50%
T. repens	Huia	32.50%
T. repens	Haku	0%
M. truncatula	Jemalong	12.5%

Figure 1 The amplification of the ban gene (1.5kb) with four different $MgCl_2$: 2.5mM, 3mM, 3.5mM, 4mM



Conclusions The next step is to sequence and clone the gene before transforming the plasmid into white clover and *Medicago truncatula* via *Agrobacterium tumefaciens* mediated transformation. As the efficiency of transformation is relatively low (0.3%-6%) for white clover (Ding *et al.,* 2003) and can be a lengthy process, it is essential not to limit transformation further by using a poorly regenerating cultivar. There exists a wide variation of successful regeneration within the white clover cultivars at Teagasc (Table 1). Only cultivars with a high level of regeneration will be used for the transformation process.

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