



Tools for use in the Genetic Engineering of Barley.

Agnelo Furtado¹, Robert Henry¹ and K. J. Scott².

¹Cooperative Research Centre for Molecular Plant Breeding, Centre for Plant Conservation Genetics,
Southern Cross University, Lismore-2480, Australia.

²The Department of Biochemistry, The University of Queensland, St Lucia-4072, Australia.

Abstract

Genetic engineering provides an opportunity to express specific trans-genes in barley. Specific promoter regions of genes can be used to control spatial and temporal expression of trans-genes in genetically engineered barley. The development of transgenic barley for commercial use depends on freedom-to-operate (FTO), with both the trans-genes and the promoters used. We are interested in applying genetic engineering to improve barley with respect to disease resistance and quality. Promoters for which we have FTO are the *prb-1* (a pathogen-inducible promoter) and the *asi* (an endosperm-specific promoter). Investigation of both these promoters indicates that they could be used to produce commercial varieties with highly desirable traits. These represent valuable tools for use in the genetic engineering of barley.

Introduction

Conventional breeding has been used to modify the genetic makeup of plants for the benefit of mankind and their domesticated animals. However, with advances in gene-transfer technology researchers have applied molecular tools for the expression of novel genes (trans-genes) to modify a range of agronomic or end use characteristics. Genetic engineered plants have been generated for various uses such as increased tolerance to disease or the generation of novel proteins for increased nutritional value (Taber L. and Higgins T. J. V. 1998), or the production of vaccines, pharmaceuticals, plastics, industrial oils etc (Taylor C.B. 1998). However, in the normal process of plant development the expression of many genes is regulated in response to internal and/or external cues. Regulation of gene expression evolved in the plant is crucial for normal metabolism, growth and plant development in general and is controlled at the transcriptional or post-transcriptional level. Transcriptional regulation of genes results from interactions of DNA binding proteins with regions on the 5'-flanking sequences

of genes also known as promoter regions. Thus using genetic engineering and the right promoter sequences the activation or suppression of trans-genes can be achieved. However, the development of transgenic barley for commercial use depends on freedom-to-operate (FTO), with both the trans-genes and the promoters used. We are interested in applying genetic engineering to improve barley with respect to disease resistance and quality. Promoters for which we have FTO are the *asi* (an endosperm-specific promoter) and the *prb-1* (a pathogen-inducible promoter).

Studies on the promoter of the *asi* gene.

The bifunctional alpha-amylase subtilisin inhibitor protein (ASI) is synthesized in developing barley grain and its expression is regulated by the hormone abscisic acid and gibberellic acid (Mundy, Svendsen *et al.* 1983). ASI-protein inhibits the high-pI group of alpha-amylases in wheat and barley (Battershell and Henry 1990) and could have a role in regulating the action of alpha-amylase (Henry, Battershell *et al.* 1992; Henry, McKinnon *et al.* 1994). In addition, ASI-protein could have a defensive role as it inhibits the bacterial protein subtilisin (Leah and Mundy 1989). Using an ELISA-based assay, *asi*-protein level as compared to total seed protein was found relatively stable under various environmental conditions in Australia. The promoter of the *asi* gene was isolated by inverse-PCR and using homology-search putative elements for hormonal and endosperm specific expression was identified. The *asi*-promoter was analyzed for promoter-strength and tissue-specific expression by transient reporter-gene expression assay as well as in transgenic rice plants. Gene constructs were designed to express the green fluorescent protein gene (*gfp*) under the control of the *asi*-promoter and its deletions. In barley the isolated *asi*-promoter confers on *gfp*, transient aleurone-specific expression (figure 1). Comparison of the *asi*-promoter to other endosperm-specific cereal promoters and ABA and GA-responsive promoters is being carried out using transgenic plants and transient reporter-gene expression assay. In rice plants transformed with the *asi-gfp.nos* construct, GFP-protein is expressed in immature leaf tissue, stigma and expression of GFP in developing endosperm is currently being investigated.

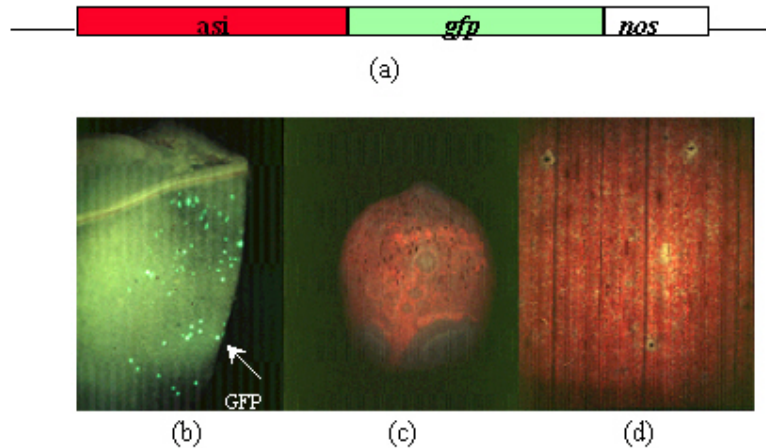


Fig 1. Aleurone-specific expression of *gfp* under the control of the *asi*-promoter.

Aleurone (b), pericarp (c) and leaf (d) tissues were bombarded with the construct *asi.gfp.nos* (a) and cells expressing green fluorescence were detected only in the aleurone layers. Green fluorescence of the GFP-protein was observed under blue light (excitation 490 and emission at 510 nm) using a compound microscope.

Studies on the *prb-1* promoter from barley

In barley the pathogenesis related genes type 1 (*pr1*) comprise a gene family of at least six genes and are expressed in response to pathogens and certain chemicals (Davidson, Manners *et al.* 1987, Muradov, Petrasovits *et al.* 1993). The *prb-1* gene is a member of this *pr1*-gene family and homologues have been reported to be present in both wheat (Molina A., EMBL: AJ007349) and rice (Bhargava, T. and Hamer, J. E., EMBL U89895). Using northern analysis transcript homologous to the *prb-1* gene was expressed in barley in response to infection by *Erysiphe graminis* f.sp. *hordei* (barley powdery mildew fungus, BPMF) and after treatment with chemical such as 2,4-dichloro isonicotinic acid (DCINA) and benzotriazole (BTH). Similar patterns of expression were obtained in wheat after treatment with DCINA, BTH and after infection with *Erysiphe graminis* f.sp. *tritici* (wheat powdery mildew fungus, WPMF). Investigation of the *prb-1* promoter and its deletions using a transient reporter-gene expression assay indicated that the promoter is not induced in response to infection with the BPMF, WPMF or treatment with DCINA or BTH. In transgenic rice plants the *prb-1* promoter was not induced after treatment with DCINA and BTH. However, in transgenic rice plants the *prb-1* promoter confers on *gfp* gene constitutive expression in the terminal cell of trichomes and induced-expression in leaf tissue around necrotic-like lesions.

Conclusions

Applications of the *asi*-promoter in barley.

The promoter of the *asi* gene could be used to express novel proteins in barley for various uses such as to improve nutritional quality of fodder or for end-use applications such as in the beer industry. The promoter could also be used for endosperm specific expression of proteins in other cereals.

Applications of the *prb-1* promoter in barley.

Study of plant microbe interaction has contributed immensely in understanding the molecular basis of resistance and susceptibility to a disease. Plants react to pathogen attack by activating an elaborate defence mechanism that acts both locally and systemically. However, resistance or susceptibility to a pathogen depends on the recognition of the pathogen followed by the timely induction of a defence response. Crop protection against pests and disease has been advanced tremendously by resistance breeding, application of pesticides and by a variety of agronomic practices such as crop rotation. Using gene transfer techniques the expression of resistance related trans-gene/s could further contribute to crop improvement by genetically modifying susceptible plants for increased resistance. Control of spatial and temporal expression of trans-gene/s in response to a range of pathogens can be achieved by using pathogen inducible promoters. Our results indicate that the promoter of the *prb-1* gene could be used to direct expression of defense genes at the site of infection for increased resistance.

References

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