AGROBACTERIUM - MEDIATED TRANSFORMATION OF HOP

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INTRODUCTION

Breeding in hops is lengthy and hindered by several obstacles, including difficulties in testing male plants, making breeding aims difficult to achieve. Gene transfer technologies are an attractive alternative to conventional breeding methods since they enable relatively rapid introduction of desirable characteristics into established hop cultivars without altering their quality profiles. Only two authors achieved stable *gus* reporter gene expression in two genetically closely related hop genotypes (1, 2). Since regeneration ability of hop is highly genotype dependent (3), a specific/modified regeneration and subsequently transformation protocol for each variety needs to be established. So far no successful transformation protocol in any Slovenian hop cultivar has been published.

MATERIAL AND METHODS

A. tumefaciens LBA4404 carrying plasmid pCAMBIA1301 (CAMBIA, Australia) provided with the intron-containing *gus* reporter gene and *hptII* selection gene, both driven by CaMV 35S promoter was used for transformation of Slovenian most widely grown hop cvs. Aurora and Savinjski golding. Internodal explants were subjected to a transformation procedure as previously described (4) and planted on hop regeneration medium (5), supplemented with either acetosyringon 100 μM for co-cultivation with *Agrobacterium* or with timentin 150 mg/l for regeneration of transformants. Approximately six months after transformation procedure emerging hop regenerants were tested for the *gus* gene expression by histochemical analysis of β-glucoronidase (GUS) activity (6). Subsequently, GUS expressing shoots were checked by PCR analysis for integration of reporter and selection genes. The primers used were designed to amplify a 408 bp fragment in the *gus* gene and a 641 bp fragment in the *hptII* gene, respectively.

RESULTS AND DISSCUSSION

Blue staining of tested shoots showed stable and not only transient reporter gene expression since GUS-assay was performed more than six months after explants were subjected to the transformation procedure. GUS staining showed GUS expression in leaves, stems and roots (Fig. 1) of transformed hop plants. According to GUS test we obtained 10.5% transformation efficiency in cv. Aurora and 26.7% efficiency in cv. Savinjski golding.

Fig. 1: Expression of gus gene in hop leaves, shoots, stems and roots

Some regenerants had most of the tissue stained blue, while with others, only small islands of cells or predominantly vascular tissue colored blue, which has already been reported in other plants (7). The intensity and pattern of blue coloration probably depends on the integration site and on the copy number of the gus gene, which is randomly integrated into the plant genome. Chimeras are another but less likely possibility.

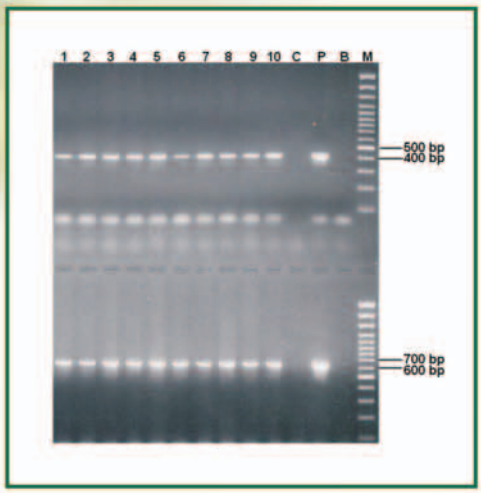

Fig. 2: DNA of GUS staining positive hop plants cv. Savinjski golding amplified with primers for gus (408 bp) and hptII (641 bp) gene, respectively: 1-10; C control plant; P purified plasmid pCAMBIA1301; B blind sample; M GeneRuler 100 bp DNA Ladder Plus

Molecular analysis by the PCR method of GUS positive regenerants demonstrated integration of both transgenes in cv. Savinjski golding (Fig. 2). In cv. Aurora we observed majority of shoots having both transgenes integrated (92.2%) and just a few shoots analyzed showed just *gus* (7.8%) gene presence. The later could be due the fact that Aurora regenerants were plated on medium without hygromycin selective antibiotic, because hop showed high sensitivity to hygromycin selection.

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