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# Activity of hygromycin phosphotransferase marker gene optimised for genetic modifications of miscanthus

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Biotechnological methods based on genetic engineering and plant transformation are essential complement of research on biomass production of miscanthus and other energy plants. Hygromycin phosphotransferase (hpt) is one of commonly used marker genes used for such manipulations. Preliminary tests showed that hygromycin B is an appropriate selection factor for miscanthus species (*Miscanthus x giganteus, M. sinensis, M. sacchariflorus*). A vector with optimised *hpt* coding sequence can be a promising tool for transformation. In order to increase its expression and consequently improve efficiency of miscanthus transformation, the coding sequence of bacterial *hpt* gene (*Streptomyces hygroscopicus*) was optimised basing on codon usage specific for maize (*Zea mays*, Codon Usage Database, www. cazusa.or.jp). Optimised *hpt* coding sequence (Ohpt) was introduced into pCAMBIA 1201 vector to replace original *hpt* gene and achieve pCAOhpt. Both vectors were used for transformation of tobacco (*Nicotina tabacum*) as a model plant. Regenerating explants were cultured on selection media containing 10, 20 and 50 mg/L of hygromycin (hyg). After transformation with pCAOhpt, plants regenerated on media with hyg 10 and 20 mg/L amounted 142% and 130%, respectively, in comparison to those obtained for pCAMBIA. In the case of hyg 50 mg/L, only pCAOhpt gave positive results (4 plants regenerated). The Ohpt sequence was detected using PCR in 50%, 83% and 100% of plants for hyg 10, 20 and 50 mg/L, respectively, while *hpt* sequence was present in 62.5% and 66% of obtained plants, for hyg 10 and 20 mg/L, respectively. Thus, it is estimated that the Ohpt sequence increases transformation efficiency by approx. 35% and decreases risk of false positive transgenic plants (escapes). Further analyses confirming functionality of t, as RT-PCR and assay of enzymatic activity of hygromycin phosphotransferase are in progress. However Ohpt sequence has been already used for contruction of vector pCAHGA, containing Ohpt sequence under control of ubiquitin1 promoter (form maize) and GUS reporter gene under control of actin1 promoter (rice) for development of miscanthus transformation method.

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