核农学报 2012, (3) 444-448,483 DOI: ISSN: 1000-8551 CN: 11-2265/S

植物诱变育种·农业生物技术

以木糖异构酶基因作为选择标记的花生遗传转化

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摘要:

用木糖异构酶基因 xylA 替换掉pCAMBIA1301中的潮霉素磷酸转移酶基因(hygromycin-B-phosphotransferase, *Hpt*),构建了植物表达载体pCAMBIA1301-xylA。再利用农杆菌介导法将pCAMBIA1301-xylA导入花生,转移到添加蔗糖(5g/L)和不同浓度木糖(5, 10, 20, 30g/L)的体胚诱导及体胚萌发培养基上培养。对外植体成苗率及转基因阳性率进行统计,结果表明,当木糖浓度为10g/L时,诱导成苗率为15.25%,再生植株生长健壮,且转基因阳性率高达77.27%,最终确定10g/L为最适木糖筛选浓度。该筛选方法利用木糖作为筛选剂,可以避免利用抗生素筛选可能造成的安全隐患,转化效率高,是一种安全、高效的筛选方法。

关键词: 花生 木糖异构酶基因 农杆菌介导法 遗传转化

THE GENETIC TRANSFORMATION OF PEANUT USING XYLOSE ISOMERASE GENE AS A SELECTION MARKER

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Abstract:

The xylose isomerase gene (xylA) was cloned into pCAMBIA1301 vector from which the hygromycin-B-phospho transferase gene (*Hpt*) had been eliminated, and was designated as the recombinant plasmid pCAMBIA1301-xylA. It was transformed into the explants of peanut embryonic leaflet by Agrobacterium -mediated method. These embryonic leaflet explants were then transferred to the somatic embryogenesis induction medium and somatic embryogenesis germination medium with sucrose (5g/L) and different concentrations of xylose (5, 10, 20, 30g/L) respectively, under the condition of 25℃, 3000lx, 13h light/11k dark. The results showed that the explants regeneration rate was 15.25%, the regeneration plants were strong and the transgenic positive rate was up to 77.27% at the concentration of 10g/L of xylose, so the 10g/L of xylose was considered to be the optimal screening concentration. The selection method using xylose could avoid the use of antibiotics which might cause security risks. The new developed method was found to be practicable, highly efficient, and safe for the genetic transformation of peanut.

Keywords: peanut xylose isomerase gene(xylA) Agrobacterium- mediated method genetic transformation

收稿日期 2011-11-17 修回日期 2012-02-21 网络版发布日期

DOI:

基金项目:

山东省中青年科学家奖励基金(BS2009NY028),国家自然科学基金(31101178, 30871544)

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