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PEG法介导转化诸葛菜下胚轴原生质体获得转基因植株

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摘要 采用诸葛菜无菌苗的下胚轴组织为材料, 分离原生质体,在原生质体培养基中作液体浅层暗培养,植板率为5%,植株再生频率为100%。作者进而开展了遗传转化研究。为研究PEG介导转化诸葛菜原生质体的影响因素,通过瞬间表达,实验了PEG法转化子叶原生质体的过程,在此基础上将分离纯化后的原生质体与带HPT基因的质粒DNA(pBI222)混合, HPT基因作选择标记,PEG介导转化;重新收集转化后的原生质体,以5×10 E4/ml的密度在原生质体培养基中作浅层培养;培养10-15天后用25mg/L的潮霉素(hygromyc in)进行筛选,一月后出现少量细胞团,转入含潮霉素50mg/L的扩增培养基扩增愈伤组织, 进而转入含50-100mg/L潮霉素的分化培养基诱导分化成苗,分化率为100%,转入生根培养基中生根成完整植株。抗性植株再生率为4×10 E-5。在获得再生转基因植株后,以再生植株叶片为材料,进行Southern blot分子杂交,证实外源基因已稳定整合到植物基因组中并表达,再生转基因植株频率为10 E-5。国内外首次转化诸葛菜属植物原生质体获得成功。

关键词 诸葛菜 下胚轴 原生质体培养 PEG法 转基因植株

分类号

PEG-medium Transformation of Orychophrage us violaceus Hypocotyl Protoplast and Regeneration of Transgenic Plants

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Abstract

Hypocotyl protoplasts were isolated with enzyme solution,and used as explants for protoplast culture.The division frequency was about 50%,the frequency of plant regeneration was 100%.The transient gene expression with cotyledon protoplasts was studied,based on the study of transient gene expression and protplast culture,The protoplasts isolated were treated with bacterial plasmid DNA (pBI222),and cultured at a density of 5x10⁴ /ml.After 10- 15 days,they were selected by adding 25<SPAN lang=EN-US style="FONT-SIZE: 10.5pt; FONT-FAMILY: 宋体; mso-bidi-font-size: 12.0pt; mso-bidi-font-family: 'Times New Roman'; mso-font-kerning: 1.0pt; mso-ansi-language: EN-US; mso-fareast-language: ZH-CN; mso-bidi-language: AR-SA">μg/ ml bygromycine. One month later, a few calli observed were transferred onto the solid proliferation medium with 50<SPAN lang=EN-US style="FONT-SIZE: 10.5pt; FONT-FAMILY: 宋体; mso-bidi-font-size: 12.0pt; mso-bidi-font-family: 'Times New Roman'; mso-font-kerning: 1.0pt; mso-ansi-language: EN-US; mso-fareast-language: ZH-CN; mso-bidi-language: AR-SA">μg/ ml hygromycine. After transferred onto the differentiation rooting medium, the hygromycine-resistant plants were obtained.The whole plants were transplanted into pots and grew well. The frequency pf regemeration was 5x10⁻⁵. The excised leaves of the transgenic plants were used as explants of Southern blot analysis. It was confirmed that HPT gene had been stably integrated into the chromosomal genome of Orychophragmus violaceus. The transformation frequency of hypocotyl protoplasts was 10⁻⁵</SPAN></SPAN>

Key words Orychophragmus violaceus Hypocoyl Protoplast culture PEG Transgenic plant

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