Research Article

The Efficiency of GFP Gene Transformation on Peanut Embryo Somatic Using Agrobacterium and Particle Bombardment

Sholeh Avivi1,*, Ralf G. Dietzgen2, Colleen M. Higgins2, Sudarsono3

1) Department of Agroecotechnology, Faculty of Agriculture, Jember University;
2) Queensland Agricultural Biotechnology Centre (QABC) Australia
3) Department of Agronomy, Faculty of Agriculture, Bogor Agriculture University

Correspondence author, e-mail: avi_vi@yahoo.com.

ABSTRACT

The aims of this research were to (1) compare the effectiveness of established Agrobacterium mediated transformation and bombardment mediated transformation on peanut (2) evaluate the integration of green fluorescent protein (GFP) and hygromycin resistance (hyg) gene on the peanut embryosomatics. To achieve those objectives we shoot 1200 explants using bombardment and transform 1000 explants using Agrobacterium mediated transformation. Both of Agrobacterium mediated transformation and particle bombardment could give positive embryos transformed. Particle bombardment gave 4.5% transformation efficiency while Agrobacterium mediated transformation gave only 1.8%. From this research, we concluded that particle bombardment more efficient and gave more transgenic explants than Agrobacterium mediated transformation.

Key words: agrobacterium, bombardment, GFP.

INTRODUCTION

Nowadays many transgenic peanut have produced using several methods such as Agrobacterium mediated transformation and bombardment mediated transformation (McKently et al. 1995; Wang et al. 1998). Here we compare the efficiency of those methods.

METHODS

This research was done at QABC-QDPI (Queensland Agriculture Biotechnology Centre-Queensland Department Primary Industry) Laboratory, Australia. We transformed peanut with bombardment (plasmid pSAQ2) as Livingstone & Birch (1996) methods and with Agrobacterium:pSAQ2 mediated transformation (Avivi, 2000; Avivi, 2009). At 11 weeks after transformation, transgenic embryosomatic was tested and evaluated its hpt and GFP using PCR as Thomson & Dietzgen (1995). The primers that used were:

Hyg Forward (HF) : 5’-AAA AGT TCG ACA GCG TCT CCG ACC-3’
Hyg Reverse (HR) : 5’-TGG GCC ACC TCG TAT TGG GAA TCC-3’
m-GFP-5’ : 5’-GAC GAC GGG AAC TAC AAG AC-3’
RESULT

We had 54 somatic embryos from 1200 embryogenic calli after bombardment transformation treatment and 18 somatic embryos from 1000 embryogenic calli after agrobacterium transformation (Table 1, Figure 2). Then we tested somatic embryos using PCR technique as Thomson & Dietzgen (1995) (Figure 3 & 4).

Table 1. Agrobacterium and bombardment transformation efficiency

<table>
<thead>
<tr>
<th>Transformation Method</th>
<th>Number of explant</th>
<th>Number of calli hyg</th>
<th>Number of embryo</th>
<th>% Embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bombardment</td>
<td>1200</td>
<td>54</td>
<td>54</td>
<td>4.5</td>
</tr>
<tr>
<td>Agrobacterium</td>
<td>1000</td>
<td>18</td>
<td>18</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Note: Data was evaluated on 11 weeks after transformation. % Embryo = (Number of embryos/Number of explant) x 100%.
CONCLUSION

The result of this research showed that particle bombardment more efficient compare with Agrobacterium mediated transformation (Table 1, Figure 2, 3, 4).
AKNOWLEDGMENT

Special thanks to supervisors Prof. Surkati Abidin, Prof. Rusmilah Suseno, Prof. Sudarsono Ph.D, Hajrial Aswidinnoor Ph.D, and Satriyas Ilyas Ph.D (IPB-Indonesia); Ralf G. Dietzgen Ph.D., and Colleen M. Higgins Ph.D, (QABC-Australia). Also to the project donor “Crawford Fund, Australia” (cq. Sholeh Avivi).

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