**Indonesian Protein Society**
Proceeding of International Seminar and Workshop on Protein
Jember, October 29-30, 2014

*Research Article*

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

**Sholeh Avivi[1,*)], Ralf G. Dietzgen[2)], Colleen M. Higgins[2)], Sudarsono[3)]**

[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 Agro bacterium mediated transformation and bombardment mediated transformation on peanut (2) evaluate the integration of green fluorescent protein (GFP) and hygromycin resistance ($hyg^r$) 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 $hpt^r$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'-TTG GCG ACC TCG TAT TGG GAA TCC-3'
m-GFP-5' : 5'-GAC GAC GGG AAC TAC AAG AC-3'

m-GFP-3′ : 5′-CAT CCA TGC CAT GTG TAA TC-3′

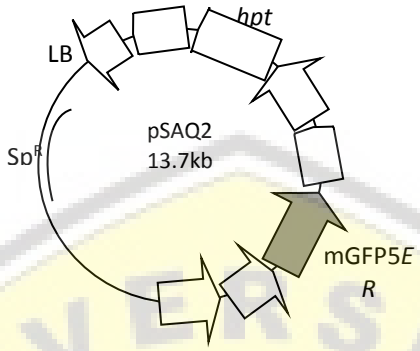


Figure 1. Mapping of pSAQ2 plasmid (Avivi, *et al*., 2009)

## RESULT

We had 54 somatic embryos from 1200 embryiogenic calli after bombardment transformation treatment and 18 somatic embryos from 1000 embryiogenic 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

| Transformation Method | Number of explant | Number of calli hyg$^r$ | Number of embryo | % Embryo |
|---|---|---|---|---|
| *Bombardment* | 1200 | 54 | 54 | 4.5 |
| *Agrobacterium* | 1000 | 18 | 18 | 1.8 |

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

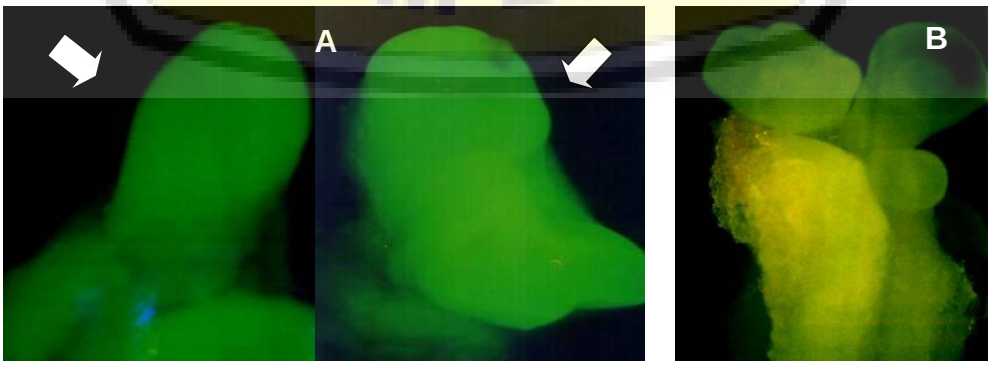


Figure 2. A. Positive GFP of peanut embryo (arrow mark) B. Control

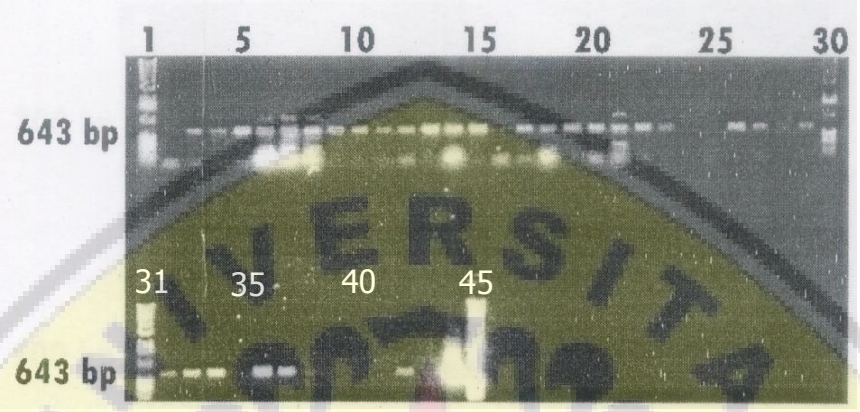


Figure 3. PCR of total DNA from a half of embryo using hygromycin primer (HF/HR). Line 1, 30, 31, and 45, are 1 kb ladder marker; Line 2, control (aquades); Line 3-22, 20 total DNA from a half of embryo after bombardment treatment; Line 21-29 and 32-42, 18 total DNA from a half of embryo after Agrobacterium treatment; Line 3-15, 17-23, 26-29, 32-34, 36-38, 42, and 44 positive embryo with resistant hygromicin gene (643 bp fragment). Line 6, 24, 25, 35, and 39-41 embryo without resistant hygromicin gene (no band); Line 43 and 44 plasmid DNA pBCSK+:mGFP5ER (maxiprep) and plasmid DNA pSAQ2 (maxiprep).

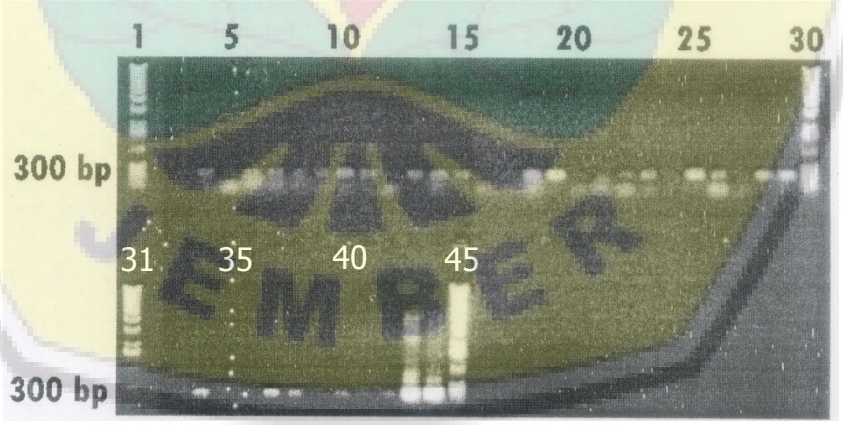


Figure 4. PCR of total DNA from a half of embryo using GFP5' & GFP3' primer. Line 1, 30, 31, and 45, 1 kb ladder marker; Line 2, control (aquades); Line 3-22, 20 total DNA from a half of embryo after bombardment treatment; Line 21-29 dan 32-42, 18 total DNA from a half of embryo after Agrobacterium treatment; Line 3-23, 25-29, 32-34, 36-38, 40 and 42-44 positive embryo with GFP gene (300 bp fragment). Line 24, 35, 39 and 41 embryo without GFP gene; Line 43 and 44 plasmid DNA pBCSK+:mGFP5ER (maxiprep) and plasmid DNA pSAQ2 (maxiprep).

## CONCLUSION

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

**AKNOWLEGMENT**

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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ISBN: 978-602-9030-88-4

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