Indonesian Journal of Agricultural Science
Vol. 17 No. 2 October 2016: 49–56
DOI: http//dx.doi.org/10.21082/ijas.v17n2.2016.p49-56

Agrobacterium tumefaciens-MEDIATED *IN-PLANTA* TRANSFORMATION OF INDONESIAN MAIZE USING pIG121Hm-Cs PLASMID CONTAINING *npt*II AND *hpt* GENES

Transformasi Tanaman Jagung Indonesia dengan Plasmid pIG121Hm-Cs yang Mengandung Gen nptII *dan* hpt *melalui* Agrobacterium tumefaciens *secara* In-Planta

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Submitted 10 May 2016; Revised 5 September 2016; Accepted 10 September 2016

ABSTRACT

Maize (*Zea mays* L.) productivity in Indonesia is challenged to be increased using genetic engineering. Recent advances in *Agrobacterium tumefaciens*-mediated *in-planta* transformation makes it possible to transform maize with low cost, and simple method. This study aimed to confirm pIG121Hm-*Cs* plasmid in *A. tumefaciens*, and to estimate the efficiency level of *A. tumefaciens*-mediated *in-planta* transformation of Indonesian maize by using pIG121Hm-*Cs* plasmid containing *npt*II and *hpt* genes. A series of studies were conducted including confirmation of gene construct of pIG121Hm-*Cs* plasmid in *A. tumefaciens*, transformation of four maize lines through *A. tumefaciens*-mediated *in-planta* technique, acclimatization of transformant plants and molecular analysis of selected plants using polymerase chain reaction (PCR). The pIG121Hm-*Cs* plasmid was confirmed via PCR analysis using specific primers of *npt*II and *hpt* genes and resulted 700 bp and 500 bp for fragments of *npt*II and *hpt*, respectively. After selection, acclimatization and molecular analysis steps, the efficiency levels of transformation of four maize lines were low, ranging from 3.8% to 12.8%. The level of transformation efficiency of ST-27 line was the highest accounting for 12.8% of 45 planted embryos on selection medium based on PCR analysis using specific primer for *npt*II gene. Overall, *A. tumefaciens*-mediated *in planta* transformation on maize floral pistil in this study proved to be successful and rapid. Therefore, this enhanced transformation method will be beneficial for Indonesian maize genetic engineering.

[***Keywords***: Maize, *Agrobacterium tumefaciens*, *in-planta* transformation]

ABSTRAK

Upaya peningkatan produktivitas jagung dapat dilakukan melalui rekayasa genetik tanaman. Proses transformasi merupakan kunci keberhasilan dalam rekayasa genetik tanaman. Metode transformasi in-planta *menggunakan* Agrobacterium tumefaciens *merupakan metode transformasi yang sederhana dan murah. Penelitian ini bertujuan melakukan konfirmasi konstruksi gen pada plasmid pIG121Hm-*Cs *di dalam* A. tumefaciens, *dan menduga tingkat efisiensi transformasi melalui* Agrobacterium *secara in planta pada tanaman jagung Indonesia dengan plasmid pIG121Hm-*Cs *yang mengandung gen* npt*II dan* hpt. *Penelitian dilaksanakan melalui beberapa tahapan secara berurutan, yaitu konfirmasi konstruksi gen pada plasmid pIG121Hm-*Cs *dalam* A. tumefaciens, *transformasi empat galur jagung melalui* A. tumefaciens *dengan teknik* in-planta, *aklimatisasi tanaman transforman, dan analisis molekuler tanaman transforman terseleksi menggunakan* polymerase chain reaction *(PCR). Keberadaan plasmid pIG121Hm-*Cs *dikonfirmasi dengan analisis PCR menggunakan primer spesifik untuk gen* npt*II dan* hpt *dan dihasilkan fragmen DNA berukuran 700 pb untuk gen* npt*II dan 500 pb untuk gen* hpt. *Setelah tahap seleksi, aklimatisasi, dan analisis molekuler, efisiensi transformasi keempat galur jagung masih rendah, berkisar antara 3,8–12,8%. Tingkat efisiensi transformasi tertinggi ditunjukkan oleh galur jagung ST-27, yaitu 12,8% dari 45 embrio yang ditanam pada media seleksi berdasarkan analisis PCR menggunakan primer spesifik untuk gen* npt*II. Berdasarkan hasil tersebut, transformasi tanaman jagung melalui* A. tumefaciens *secara* in-planta *pada putik bunga terbukti berhasil dan cepat. Metode transformasi ini akan bermanfaat untuk rekayasa genetik pada tanaman jagung.*

[***Kata kunci***: Jagung, *Agrobacterium tumefaciens*, transformasi *in-planta*]

INTRODUCTION

Maize (*Zea mays* L.) is a cereal crop that is widely grown and has the highest productivity in the world. This crop is usuallay used as food, feed and industrial raw materials of maize flour, refined products, as well as industrial fermentation and distillation (Wada *et al*. 2008). In Indonesia, maize is an important food commodity after rice and soybean.

Maize productivity is strongly influenced by biotic and abiotic stresses (Rakshit *et al.* 2009). Genetic improvement of maize to increase its productivity can be conducted by conventional hybridization between popular variety with other variety containing desired genes. These desired genes will be used for plant improvement through non-conventional technique or genetic engineering by transformation of homologous or heterologous genes which have been used for varied purposes (Carneiro and Carneiro 2013). Inserted genes (foreign genes) can be derived from the relative taxon or cross-taxon such as bacteria, fungi, viruses or distant relatives of crops regarding the genes of interest (Herman 1997).

The conventional plant transformation is a biological transfer method through *Agrobacterium* and physical or chemical gene transfer like gene gun to produce plants containing desired traits. Utilization of *A. tumefaciens* in plant genetic engineering is based on its ability to transfer gene that causes crown gall disease in plant cells. This phenomenon is exploited by researchers to replace "the crown gall disease" genes with genes encoding the desired properties so that these genes can be integrated into the plant genome and expressed by plants. The main step in *A. tumefaciens* method is based on co-incubation of plant vegetative organ or tissue (seeds, leaves, roots, stems or meristems) in bacterial-cell suspension (Chumakov 2007). These methods have been successfully used for transformation of important genes to many plants whose tissue culture system has already been established (Hiei *et al.* 1994; Ishida *et al.* 1996; Gelvin 2003; Carneiro and Carneiro 2013; Martins *et al.* 2015; Jan *et al.* 2016). Some advantages of transformation via *A. tumefaciens* have been reported such as higher transformation efficiency with a single copy gene and can be carried out with simple laboratory equipment and low-cost technique (Supartana *et al.* 2005). Transformants with a single copy gene are preferable as it is a easest analysis process and the segregation usually follows Mendelian inheritance pattern (Rahmawati 2006). But, the tissue culture-based transformation methods are time-consuming and lead some mutations from clonal variation that affect both qualitative and quantitative characters of plants (Martins *et al.* 2015; Jan *et al.* 2016).

The direct transformation method without any tissue culture steps is termed as *in-planta* transformation (Chumakov *et al.* 2006). Floral dip method has been used in genetic engineering strategy as it directly produces genetically modified transformant without the laborious tissue culturing procedures (Martins *et al.* 2015). The production of a large number of uniform plants in a short time, less labor efforts and minimal reagent requirements are some of the main advantages of *in-planta* transformation system (Bent 2000). Production of transformants *in-planta* seems especially promising for monocotyledonous plants, including maize since plant regeneration from suspension and callus cultures is difficult (Moiseeva *et al.* 2014). This method also proved to be faster, simpler and cheaper (Mu *et al.* 2012).

The development of a simple and rapid transformation method for *Setaria viridis* as a C4 model for monocotyledonous plants was time-saving and cost-effective compared with traditional methods in plant genetic engineering (Martins *et al.* 2015). *In-planta* transformation techniques have been succeeded in buckwheat plant (*Fagopyrum esculentum* M.), mulberry (*Morus alba* L.) and kenaf (*Hibiscus cannabinus* L.) (Ping *et al.* 2003; Kojima *et al.* 2004). The transformation efficiency of all plants transformed by such method was extremely high compared with the general *in vitro* transformation method (Kojima *et al.* 2000).

Several studies reported the success of transformation of *npt*II gene. Supartana *et al.* (2005) obtained the levels of efficiency of *in-planta* transformation of 40% (PCR) and 34% (GUS histochemical test) by inoculation of embryonic apical meristem rice plants. *In-planta* transformation using filament pistil (female flowers) of maize with plasmid pTd33 containing *gus* and *npt*II genes showed that 6.8% sprouts from the seeds were resistant to kanamycin and 60.3% of the resistant gene *npt*II were positive (Chumakov *et al.* 2006). Other study revealed that all tested genotypes of sugarcane cv. Co86032, Co09014, CoC671, Co94012 and Co86056 responded favorably, though CoC671 proved to be the best responding cultivar with 45.4% transformation efficiency. The results concluded that the protocol is cost-effective, efficient and genotype-independent without an involvement of any tissue culture procedures and can generate a relatively large number of transgenic plants in a short period, approximately 2 months (Mayavan *et al.* 2013).

In Indonesia such transformation method has not been reported yet on maize. Therefore, the aims of this study were to confirm pIG121Hm-*Cs* plasmid in *A. tumefaciens*, and to estimate the efficiency of *A. tumefaciens*-mediated *in-planta* transformation of Indonesian maize using pIG121Hm-*Cs* plasmid containing *npt*II and *hpt* genes.

MATERIALS AND METHODS

Plant Materials and Plasmid

Four lines of maize, Ultra Genjah, ST-08, ST-18 and ST-27, were used as plant materials. *A. tumefaciens* EHA101 containing the binary pIG121Hm-*Cs* plasmid was used as gene material to be transformed (Fig. 1). The T-DNA of the pIG121Hm-*Cs* plasmid containing *npt*II gene with nos promoter (Pnos) and nos terminator (Tnos), *CsNtir-1* gene, was driven by 35s promoter and stopped by Tnos terminator, also *hpt* gene was driven by 35s promoter and stopped by Tnos terminator. The binary pIG121HM-*Cs* plasmid was the modification of pIG121Hm plasmid by replacing *gus* gene with *CsNtir-1* gene.

Confirmation of pIG121Hm-*Cs* Plasmid in *A. tumefaciens*

A single colony of *A. tumefaciens* EHA101 containing pIG121Hm-*Cs* plasmid was multiplicated on yeast extract and peptone (YEP) medium containing 50 mg l^{-1} kanamycin and incubated for two days in an incubator shaker at room temperature. Plasmid was isolated using a modified alkaline lysis method (Sambrook *et al.* 1989). PCR analyses were performed using specific primers for the *npt*II gene (nptF 5'-GAGGCTATTCGGCTATGACTG-3' and nptR 5'-ATCGGGAGCGGCGATACGTA-3') to amplify a 700 bp DNA fragment, and specific primer of *hpt* gene (hptF 5'-CGCACAATCCCACTATCCTTCGCAA-3' and hptR 5'-GGCAGTTCGGTTTCAGGCAGGTCTT-3') to amplify a 500 bp DNA fragment. PCR reaction was performed according to the protocols of Anuradha *et al*. (2006) and Listanto *et al.* (2009) with modification. Total volume of PCR reaction was 25 microliter (µl) per reaction containing 1 PCR buffer, 1.5 mM $MgCl_2$, dNTPs (each 0.2 mM dNTPs); 2 pmol primer F and R each, 0.25 units *Taq* DNA polymerase (Invitrogen), and 50 ng DNA template. The amplification reaction was done on PCR machine (MJ-Research PCT-100). Amplification cycle was started in initial denaturation at 94° C for 5 minutes followed by 30 cycles consisting of a denaturation at 94° C for 1 minute, annealing at 53° C for 1 minute and an extension at 72° C for 1 minute, then final extension at 72° C for 5 minutes. The amplification products were electrophoresed on a 1.2% agarose gel and then visualized under UV transilluminator (Chemidoc).

Agrobacterium tumefaciens-Mediated *In-Planta* Transformation on Maize

In-planta transformation was done on four maize lines (Ultra Genjah, ST-08, ST-18 and ST-27) according to the method of Chumakov *et al.* (2006). The whole pistil of a single ear of maize on 50–60 days after planting and has not pollinated was rubbed with wetted cotton containing the suspension of *A. tumefaciens* with pIG121Hm-*Cs* plasmid. The treated pistil was then dusted with pollen from the same plant and covered with paper bags. A number of maize ears were harvested on 12–16 days after pollination. The whole embryos were isolated from each maize ear and grown on MS medium without growth regulator. The embryos were then planted on MS medium in Petri dishes containing 20–30 embryos each dish and incubated at 24 °C room. After one week incubation, the embryos were transferred to MS selection medium

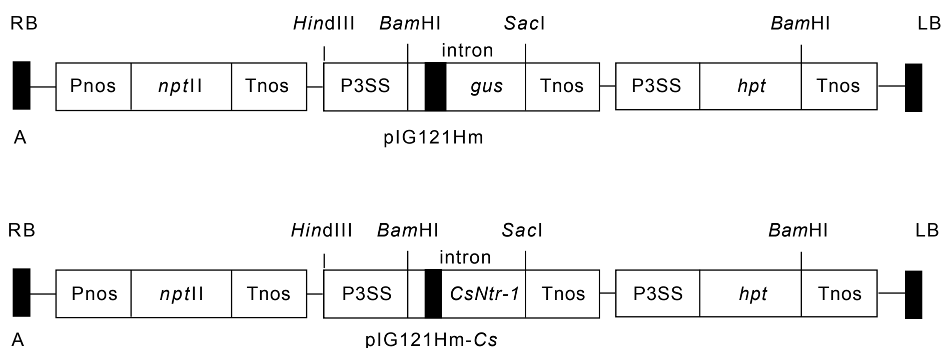

Fig. 1. T-DNA construction on the binary pIG121Hm (A) and pIG121Hm-*Cs* (B) plasmids: RB = right border; LB = left border; Pnos = promoter of the *nophaline synthase* gene; *npt*II = gene for *neomycin phosphotransferase*; Tnos = terminator of the *nopaline synthase* gene; P35S = promoter of the CaMV 35S RNA gene; *gus* = gene for *β-glucuronidase*; *hpt* = gene for *hygromycin phosphotransferase*, *CsNtr-1* = gene for nitrites transporter (Sato and Takamizo 2006).

(Murashige and Skoog 1962) containing 15 mg l^{-1} hygromycin. The percentage of survived embryos on selection medium was scored after 7 days incubation.

Acclimatization of Transformants and Molecular Analysis

Transformants which were able to grow on the selection medium (MS medium containing hygromycin) were acclimatized by transferring from *in vitro* medium into water medium. Selected transformant was chosen from a plant which was survived in the water medium and the number of survival plants in the water medium were counted.

DNA was isolated using modified method of Dellaporta *et al*. (1983). A small part of leaf (size about 0.5 cm^2) of each plant was taken, ground and homogenized in a 1.5 ml tube. Four hundred microliters (µl) of extraction buffer (Tris-HCl, pH 8.0 50 mM EDTA, pH 8.0 10 mM NaCl 100 mm, and 1% SDS) was heated to 65° C and added to the tube and shaken for 10 minutes. About 400 µl of Chisam (chloroform : isoamilalkohol = 24 : 1) solution was added and mixed thoroughly. Subsequently, the solution was centrifuged at 12,000 rpm for 10 minutes. About 250 µl of the aqueous solution was transferred to the new 1.5 ml tube, added 150 µl isopropanol and mixed by inverting the tubes for several times. DNA was precipitated by centrifugation at 12,000 rpm for 10 minutes to obtain a DNA pellet. Supernatant was discarded and the DNA precipitate was washed with 70% ethanol and dried. Dried DNA was dissolved using ddH_2O for PCR analysis.

Molecular analysis of the transformant was done by PCR amplification for the presence of *npt*II gene (one part of T-DNA component inside pIG121Hm-*Cs* plasmid) using specific primers (nptIIF and nptIIR). PCR results were electrophoresed on a 1.2% agarose gel and visualized under UV transilluminator (Chemidoc). The positive transformant was detected by the presence of DNA fragment of about 700 bp lengths in the agarose gel under UV transilluminator.

RESULTS AND DISCUSSION

Confirmation of pIGI121Hm-*Cs* Plasmid in *A. tumefaciens*

Selection of bacteria strain and binary plasmid determined the success of plant transformation using *Agrobacterium* system. *Agrobacterium* EHA101 is a highly virulent strain and therefore, it is expected to increase the rate of infection on plants. While the use of binary plasmid pIG121Hm-*Cs* can expand the infected host plants (Rahmawati 2006). In our study, *A. tumefaciens* strain EHA101 containing the binary plasmid pIG121Hm-*Cs* expectedly improved the efficiency of *in-planta* transformation on maize.

The presence of selectable marker (*npt*II and *hpt*) genes in the pIG121Hm-*Cs* plasmid in *A. tumefaciens* was confirmed by PCR using specific primers. The PCR amplification products of pIG121Hm-*Cs* plasmid in *A. tumefaciens* showed the presence of *npt*II and *hpt* genes with fragment sizes of 700 and 500 bp, respectively (Fig. 2). This confirmation result indicates that the colony of *A. tumefaciens* used for transformation contains *npt*II and *hpt* genes.

Agrobacterium tumefaciens-Mediated *In-Planta* Transformation on Maize

Three maize ears have been harvested from Ultra Genjah and ST-08 lines, while from ST-18 and ST-27 lines were got two ears on 12-16 days after pollination. After ear isolation, the number of embryos that can be isolated were 293 embryos of Ultra Genjah line, 190 embryos of ST-18, 27 embryos of ST-27 and 233 embryos of ST-08. The percentage of the number of embryos selected on MS medium containing hygromycin were 26.8% of Ultra Genjah line, 32.6% of ST-18 line, 35.9% of ST-27 line and 26.6% of ST-08 line (Table 1). The resulted germination rates were lower compared with the previous research on maize using *Agrobacterium*-mediated transformation by *in-planta* method. *In planta* floral dip transformation on maize using pCambia 1302 plasmid showed that of 79 T0

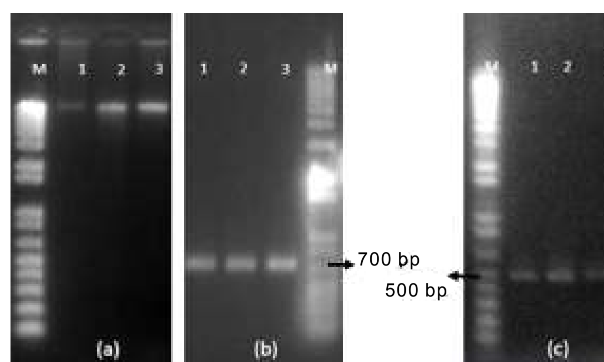

Fig. 2. Detection of marker genes (*npt*II and *hpt*) inside pIGIHm-*Cs* plasmid by PCR amplification, (a). Line 1-3: plasmid DNA, (b). Line 1-3: *npt*II DNA fragment size of 700 bp, (c). Line 1-3: *hpt* DNA fragment size 500 bp. M. 1 Kb DNA Ladder.

Table 1. The results of *Agrobacterium tumefaciens* transformation on four maize lines by *in-planta method.*

Line	Average number of planted embryos	Average number and percentage of survived embryos to hygromycin	Average number and percentage of survived plants to acclimatization	Average number and percentage of positive plants containing *npt*II
Ultra Genjah	97.7	26.0 (26.6)	2.7 (10.4)	1.0 (3.8)
ST-18	95.0	29.3 (30.8)	5.9 (20.1)	1.7 (5.8)
ST-27	22.5	14.8 (65.8)	5.0 (33.8)	1.9 (12.8)
ST-08	77.7	20.7 (26.6)	4.3 (20.7)	1.7 (8.2)

Numbers in parentheses are percentages.

seeds used for screening hygromycin resistance, the germination rate was 38% on hygromycin solution at a concentration at 20 mg l^{-1} (Mu *et al*. 2012). The low germination rate was possibly due to the seed maturity, pathogen on seed and mutation.

The pIG121HM-*Cs* plasmid had T-DNA containing selectable marker of *npt*II and *hpt* genes. A selectable marker was introduced to differentiate transformed cells from a population of untransformed cells and typically cotransformed with the gene of interest. Most selectable markers were antibiotics, such as kanamycin and hygromycin, or herbicides, such as glyphosate and phosphinothricin acethyl transferase (PAT). Kanamycin or hygromycin selection system has been used in the early step of maize transformation. Maize cells are less sensitive to kanamycin and the typically used concentration of kanamycin is 50 to 200 mg l^{-1} on maize selection, but more sensitive to hygromycin. One of the factors that contributed to the ineffectiveness of this system might be the high endogenous resistance to kanamycin of maize tissues (Ji *et al*. 2013). The hygromycin concentration used in this research was 15 mg l^{-1} for transformant selection. The concentration of hygromyin was based on the result of preliminary study that the LC_{50} (lethal concentration that kills the test embryo) was 15 mg l^{-1} hygromycin.

Acclimatization of Transformants and Molecular Analysis

Transformants which are survived on selection MS medium containing hygromycin was transferred to aqueous media for acclimatization step before being transferred into soil media. The number of transformants survived from the acclimatization process is shown in Table 1. Ultra Genjah line had 2.7 plants (10.4 % of survived embryos to hygromycin) survived in aqueous media from 97.7 planted embryos. The ST-18 line revealed 5.9 survived plants (20.1 % of survived embryos to hygromycin) from 95.0 planted embryos, and the ST-27 line showed 5.0 survived plants (33.8 5 of survived embryos to hygromycin) from 22.5 planted embryos (20.4%). Approximtely 4.3 plants of the ST 08 line survived out of 77.7 planted embryos (20.7 % of survived embryos to hygromycin). Based on the percentage of survival plants on acclimatization step, ST-27 line had the highest transformation efficiency about 33.8 % from an average of 22.5 planted embryos and Ultra Genjah line showed the lowest efficiency of 10.4 % from the mean average 97.7 planted embryos. The result of transformation and acclimatization were showed in Fig. 4.

The success of acclimatization was influenced by internal and external factors. Internal factors included less development of wax/cuticle layer, slight number of palisade cells, less development of vascular tissue from the roots to the shoots and minimum lignification of stem, while external factors were growth medium, humidity, light, temperature and pathogens (Gunawan 1992). The other factors affecting acclimatization were medium treatment, pot type and humidity (Pathi *et al.* 2013). A small pot composed of a mixture of peat moss, vermiculite and sand in the ratio of 1:1:1, and the increasing humidity by covering with polyethylene bags with small hole resulted in good plant regeneration.

One technique for early detection of transformation was the PCR amplification of the presence of the *npt*II and *hpt* genes that inserted on T-DNA of the pIG121Hm-*Cs* plasmid. T-DNA as a part of a binary plasmid (pIG121Hm-*Cs*) will be transferred through *A. tumefaciens* into plant cells by a special mechanism that involves some *vir* genes on the plasmid (Gelvin 2003). The *npt*II and *hpt* genes were introduced to differentiate transformed cells from a population of untransformed cells (Ji *et al*. 2013). Molecular analysis of transformants was done by PCR amplification of selectable marker gene using the specific primer of the *npt*II gene. Positive plants

containing *nptI*I gene were observed by the appearance of DNA fragment of 700 bp (Fig. 5). The T-DNA construction of pIG121Hm-*Cs* plasmid is composed of RB, *npt*II gene cassette, *CsNtr-1* gene cassette and *htp* gene cassette. The high frequency of gene fusions observed after T-DNA integration into plant genome, the right T-DNA border were preferentially integrated into potentially transcribed genomic loci in different plant species (Mayerhofer *et al*. 1991). Based on this study, PCR analysis for the existence of *npt*II gene fragment proved T-DNA integration of pIG121Hm-*Cs* plasmids and the other gene should be inserted into plant genom.

The percentage and number of transformants containing *npt*II gene which survived on hygromycin and acclimatization were one plant of Ultra Genjah line (3.8%), two plants of ST-18 line (5.8%), two plants of ST-27 line (12.8%) and two plants of ST-08

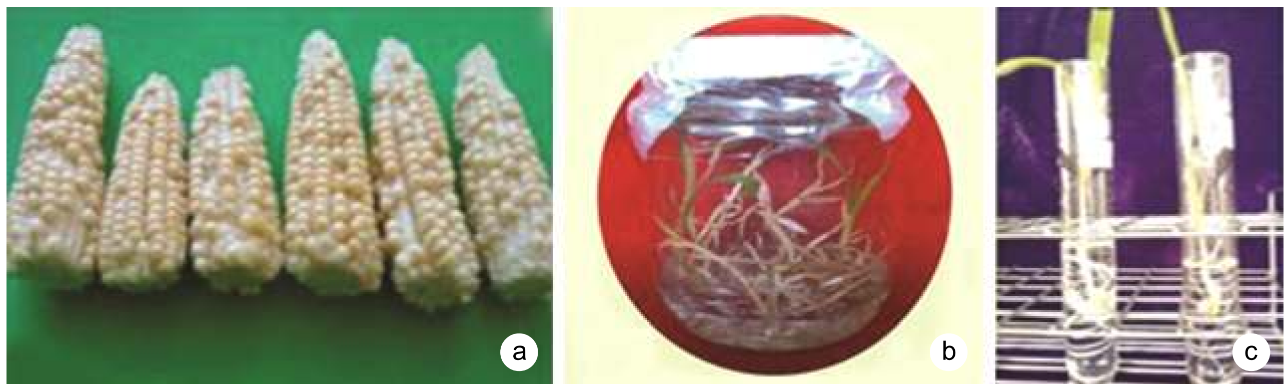

Fig. 4. *In-planta* transformant of maize: (a) *in-planta* transformant corn cob, (b) selected transformant on MS medium containing hygromycin (14 days), (c) survival plantlets on water medium (7 days), where plantlet with normal leaves was survived acclimatization.

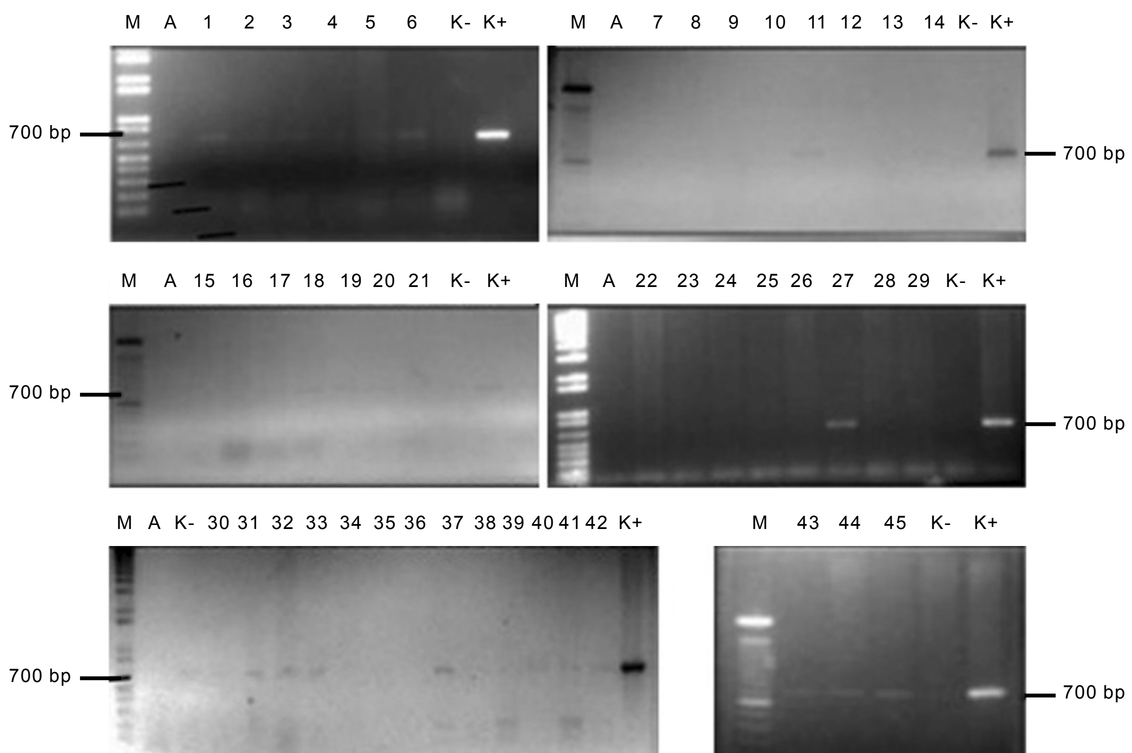

Fig. 5. PCR products of transformant using *npt*II primer (700 bp): M = 1 Kb DNA ladder, A = ddH_2O, lines 1–45 = transformants, K - = negative control (nontransformant plant), K + = positive control (containing *npt*II gene).

line (8.2%). Previous study on *in-planta* transformation showed that 60.3% lines were positive of the total of survived plants on kanamycin selection or 6.8% of the total plants tested (Chumakov *et al.*2006). In this study, ST-27 line produced 2 plants (12.8%) containing positive PCR *npt*II gene out of 15 plants survived on hygromycin selection (Table 1). The ST-27 line had the highest level of transformation efficiency, about 12.8% from 22.5 embryos planted on selection medium. On wheat, 26% PCR positive plants were obtained of the total of kanamycin-resistant plants (Razzaq *et al*. 2011). While on rice, the efficiency of transformation of T1 generation was 40% (Supartana *et al*. 2005).

Transformation efficiency resulted in this study was lower than that of other studies, and possibly caused by genetic factors of plant genotype and other factors such as temperature when treated with *Agrobacterium.* Among four maize lines studied, ST-27 had an efficiency level of 12.8% from 22.5 planted embryos. The increasing number of ST-27 line demonstrated the linear correlation with the number of transformant plants. It is assumed that the genetic background of Indonesian maize lines differs from the maize line as reported by Cumakov *et al*. (2006). Therefore, to increase the number of transformants of Indonesian maize lines, the number of embryos should be increased.

The temperature range of 19 to 22° C is optimal for the expression of *vir* genes in *Agrobacterium* (Fullner and Nester 1996) and 28° C is critical for excretion and assemblage of vir-dependent *Agrobacterium* T-pili for successful T-DNA transfer (Fullner 1998). The treatment with *Agrobacterium* suspension on the silk of maize needs specific temperature particularly above 25° C (about 09.00 AM), in constrast, temperature of 22–25° C were less favorable for *Agrobacterium*-mediated transformation. These conditions seem related to the low efficiency of *in-planta* transformation. Moiseeva *et al*. (2014) stated that temperature was the most factor in *Agrobacterium*-mediated plant transformation, including on maize with antisense suppressor of the proline dehydrogenase gene (ASPG).

Based on the results, *in-planta* transformation through *A. tumefaciens* can be done succesfully on Indonesia maize using pistil flower with minimum tissue culture step, simple and faster than regular transformation. The T_1 plants successfully survived on the selection medium containing hygromycin, showed positive PCR using primers for *npt*II gene and successfully acclimatized. It proved that maize transformation using *A. tumefaciens* can be done by *in-planta* with minimum tissue culture steps for plants those are difficult to be *in vitro* regenerated.

CONCLUSION

Maize plant transformation can be conducted using *A. tumefaciens*-mediated *in-planta* method with minimum tissue culture step. The ST-27 line had the highest level of transformation efficiency of 12.8% of the total number of embryos based on PCR analysis using specific primer for *npt*II gene. Enhancement of the number of planted embryos can increase the number of transformant plants. *A. tumefaciens*-mediated *in-planta* transformation on maize using flower pistil was faster, simpler and low-cost method.

ACKNOWLEDGEMENT

The authors highly appreciated to Miss. Retno Maryam Khodijah, alumni of the Faculty of Agriculture, Gadjah Mada University who has been involved in *Agrobacterium tumefaciens*-mediated i*n-planta* transformation. We also thank to Dr. M. Azrai and Dr. Andi Takdir, Indonesian Cereals Research Institute-IAARD, Maros, South Sulawesi for providing seed used in this study. The research was a part of Maize Research Project financially supported by governmental funding under ICABIOGRAD.

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