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Original Paper

Development of transgenic plants in jatropha with drought tolerance

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Abstract The seed oil of jatropha (*Jatropha curcas* L.) is a source of biodiesel fuel. Although jatropha can grow in semi-arid lands unsuitable for the food production, its oil productivity in such conditions is unsatisfactory at present. Therefore, it is desirable to improve the oil productivity of jatropha even in semi-arid lands by enhancing its drought tolerance. Genetic engineering is promising to dramatically improve plant traits. Although we previously reported a transformation method, which involves wounding of tissue explants in order to increase the chance of *Agrobacterium* infection, for jatropha, it remains a challenge to enhance the shoot regeneration and root induction processes. Here, we report the generation of three kinds of transgenic jatropha plants in an attempt to improve their drought tolerance. The first one overexpresses the *PPAT* gene, which encodes an enzyme that catalyzes the CoA biosynthetic pathway; the second overexpresses the *NF-YB* gene, which encodes a subunit of the NF-Y transcription factor; and the last overexpresses the *GSMT* and *DMT* genes, which encode enzymes that catalyze production of glycine betaine. We also report a modified protocol that improves the efficiency of shoot regeneration and root induction in transgenic jatropha plantlets.

Key words: Drought tolerance, *Jatropha curcas* L., transgenic jatropha.

The utilization of plant biofuels is a promising solution to problems such as the depletion of oil resources and climate change. However, the production of plant biofuels sometimes competes with the food production, potentially raising food prices (Mitchell 2008). Therefore, it is desirable to develop biofuel crops that do not compete with the food production. The seed oil of jatropha (*Jatropha curcas* L.) is a source of good-quality biodiesel fuel (BDF). Moreover, the production of jatropha oil is not thought to compete with the food production because jatropha is not an edible plant and can grow in semi-arid lands unsuitable for the cultivation of food crops. However, the productivity of jatropha oil in semi-arid lands is not commercially satisfactory at present, and as oil prices rise, it may become economic to produce jatropha in areas that are currently utilized as croplands (Li et al. 2010). Breeding jatropha with higher drought tolerance and high oil productivity even in semi-arid lands is desirable to harness the advantage of this promising plant. Therefore, enhancing the drought tolerance of jatropha is essential to improving oil productivity in semi-arid lands.

Genetic engineering is a promising and feasible way to dramatically improve plant traits. However, the application of genetic engineering to jatropha is relatively new. Thus, the transformation method for this plant is not fully established yet. One of the key challenges in successfully obtaining transgenic jatropha plants is the improvement of transformation efficiency. Initial transformation experiments conducted by

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our group employed the method of Li et al. (2008) wherein tissue explants are simply incubated with the *Agrobacterium* cell suspension. Unfortunately this procedure produces extremely low transformation efficiency. Thus, an improved transformation method that includes wounding of tissue explants to facilitate the greater chances of *Agrobacterium* infection was established (Khemkladngoen et al. 2011b). In the process of establishing a transformation method for jatropha, the regeneration of shoots from tissue explants also proved to be challenging. Despite the fact that there are several existing reports on shoot regeneration from different tissues of jatropha including our own work (Khemkladngoen et al. 2011a), regeneration conditions such as hormone concentrations and the timing of subjecting explants to certain growth hormones should be considered carefully. The step of root induction is also challenging because the resultant rate is usually low.

In this study, we report the generation of three kinds of transgenic jatropha plants in order to improve drought tolerance. The first one overexpresses the *Arabidopsis* (*Arabidopsis thaliana*) *PPAT* gene, which encodes phosphopantetheine adenylyltransferase that catalyzes the penultimate step in the CoA (CoA) biosynthetic pathway. The second overexpresses the *NF-YB* gene of *Arabidopsis* encoding the B subunit of the nuclear factor Y (NF-Y) transcription factor. The last overexpresses the *Synechococcus GSMT* and *DMT* genes encoding glycine sarcosine methyltransferase and sarcosine dimethylglycine methyltransferase, respectively, both of which catalyze glycine betaine production. Transgenic *Arabidopsis* plants that overexpress either of these gene sets exhibit improved drought tolerance (Rubio et al. 2008; Nelson et al. 2007; Waditee et al. 2005). PPAT improves drought tolerance by enhancing metabolic activitiy; NF-YB enhances the response to the drought stress by affecting the expressions of downstream genes; and glycine betaine diminishes drought stress as an osmolyte. Because it is reasonable to expect that these three means of improving drought tolerance are applicable to plant species other than *Arabidopsis*, drought-tolerance in jatropha should be enhanced by generating transgenic jatropha plants overproducing the aforementioned enzymes. We also report a modified protocol of our transgenic method, which improves the efficiency of shoot regeneration and root induction in transgenic jatropha plantlets.

Materials and methods

Plant materials and preparation of explants

Juvenile cotyledons and young leaves from jatropha (*Jatropha curcas* L.) of the Philippine, Thai, and Tanzanian lines were used for transformation. Seeds of the Philippine line were provided by University of the Philippines Los Baños, and those of Thai and Tanzanian lines were provided by Arid Land Research Center, Tottori University. The cotyledons were prepared as described in Khemkladngoen et al. (2011a). Alternatively, leaves were sterilized for 10 min in 10% Sodium hypochlorite and 0.1% Tween 20, and rinsed 3 times with sterile distilled water. The sterilized leaves were cut into squares approximately 0.5×0.5 cm. Leaf pieces were preconditioned on MS basal medium (Murashige and Skoog 1962) supplemented with 3% sucrose, 10 mg·l^{-1} thiamine, 100 mg·l^{-1} myo-inositol, 0.075 mg·l^{-1} 3-Indolebutyric acid (IBA), 1 mg·l^{-1} 6-benzylaminopurine (BA), and 0.8% agar for 2 days at 25°C.

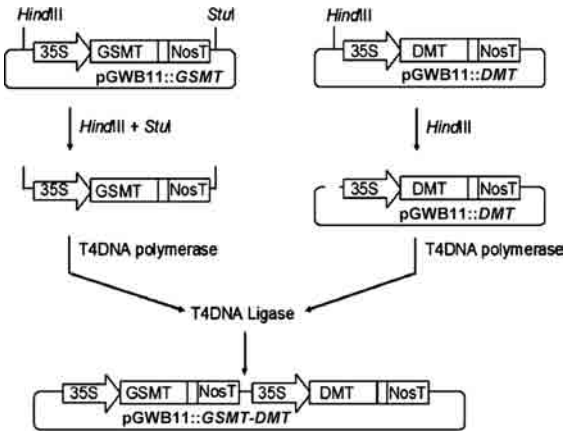

Figure 1. Construction of pGWB11::*GSMT-DMT*.

Plasmid construction and transformation of Agrobacterium

The coding sequences of *PPAT* and *NF-YB1*, and *GSMT* and *DMT* genes were amplified from the genomic DNA of *Arabidopsis thaliana* and *Synechococcus* sp. WH8102 by polymerase chain reaction (PCR), respectively. The PCR fragments were cloned by using the Gateway cloning system (Invitrogen, USA), and plasmids were constructed by using pGWB11 (Nakagawa et al. 2007)—a Gateway binary vector containing a FLAG expression cassette under the control of the cauliflower mosaic virus (CaMV) 35S promoter and the *NOS* terminator. Because 2 genes, *GSMT* and *DMT*, should be introduced together into the plant to produce glycine betaine, 3 kinds of plasmids were constructed; plasmids for co-transformation contain either *GSMT* or *DMT* alone (pGWB11::*GSMT* or pGWB11::*DMT*), whereas a plasmid for individual transformation contained both of the genes (pGWB11::*GSMT-DMT*). The pGWB11::*GSMT-DMT* plasmid was constructed from pGWB11::*GSMT* and pGWB11::*DMT* as shown in Figure 1. *Agrobacterium tumefaciens* LBA4404 was transformed by the plasmids by electroporation, and the resulting *Agrobacterium* strains were used for the plant transformation.

Agrobacterium-mediated transformation and plant regeneration systems

Agrobacterium-mediated transformation was performed

as described previously (Khemkladngoen et al. 2011b), with some modifications. Physical wounding by sonication (1 min) followed by shaking (9 min) was applied to explants during *Agrobacterium* infection. After transformation, the cotyledonary explants were cultured and regenerated using the plant regeneration system as described by Khemkladngoen et al. (2011b). The shoot regeneration medium was MS basal medium supplemented with 0.5 mg·l^{-1} IBA, 3 mg·l^{-1} BA, 20 mg·l^{-1} kanamycin (Kan), and 200 mg·l^{-1} cefotaxime. On the other hand, because of less regenerative potential and differences in the endogenous hormones in the tissues, leaf explants were regenerated with some modifications to the shoot regeneration medium. After transformation, leaf explants were cultured on shoot regeneration medium-I (SR-I) containing MS basal medium supplemented with 0.075 mg·l^{-1} IBA, 1 mg·l^{-1} BA, 0.5 mg·l^{-1} thidiazuron (TDZ), 20 mg·l^{-1} Kan, and 200 mg·l^{-1} cefotaxime for 2 weeks for shoot induction. Explants were subsequently transferred to shoot regeneration medium-II (SR-II, MS basal medium supplemented with 0.5 mg·l^{-1} IBA, 3 mg·l^{-1} BA, 20 mg·l^{-1} Kan, and 200 mg·l^{-1} cefotaxime) for shoot multiplication. We carried out shoot elongation and root induction using a modified version of the protocol described in Khemkladngoen et al. (2011b): Gamborg's B5 basal medium (Gamborg et al. 1968) supplemented with 100 mg·l^{-1} myo-inositol, 10 mg·l^{-1} thiamine-HCl, 2% sucrose and 0.2 mg·l^{-1} IBA was used as the root induction medium.

Molecular analysis of putative transformed plantlets

Total genomic DNA was isolated from the young leaves of putative transgenic and non-transgenic shoots using the DNeasy Plant Mini kit (QIAGEN, Netherlands), according to the manufacturer's protocol. PCR analysis was performed with the isolated genomic DNA to check for the presence of transgenes in putative transformants using primer pairs that amplify each transgene. Primer sequences are listed in Table 1. DNA samples were amplified using Go Taq Green Master Mix (Promega, USA). PCR was performed according to our standard protocol: initial denaturation at 95°C for 2 min; 35 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 40 s; and a final extension step of 5 min at 72°C. PCR products were separated by gel electrophoresis on 1% agarose gels, stained with ethidium bromide and visualized with a UV transilluminator.

Table 1. Primers used for PCR.

Primer	Sequences (5′→3′)
AtPPAT-F	GCTCCGGAAGATTCAAAGAT
AtPPAT-R	TCTCAGCCTCCATTTTTCTC
35S-F	AGATTAGCCTTTTCAATTTCAGAAAGAATGCTAAC
AtNFYB-R	CCAGCTCGGCATTTCTTCACCAGAAA
GSMT-F	TCTCCTGAATCGGTTCGCGAGAC
GSMT-R	GCGGTGATCAAGAATCAAGATGCC
DMT-F	GACGCTGATCAGTTTTACGAACAGG
DMT-R	CAGCAATCCCATCTCCACACCA

Quantitative analysis of glycine betaine

For quantitative analysis of glycine betaine in transgenic plants, the amount of glycine betaine in plants was measured by capillary electrophoresis-mass spectrometry (CE-MS) as described in Yu et al. (2009).

Results and Discussion

Generation of PPAT-overexpressing plants

CoA (CoA) is an indispensable cofactor in all living organisms; it functions as an acyl group carrier and carbonyl-activating group in a number of biosynthetic, degradative and energy-yielding metabolic pathways, including the oxidation of fatty acids, carbohydrates and amino acids. Phosphopantetheine adenylyltransferase (PPAT) catalyzes the penultimate step in the CoA biosynthetic pathway (Kupke et al. 2003). Recently, the action of the *PPAT* gene of *Arabidopsis* (*AtPPAT*) was analyzed (Rubio et al. 2008). The knockdown mutant of the gene exhibited severely impaired growth and seed production. In contrast, *AtPPAT*-overexpressing lines eexhibited approximately 1.6-fold higher CoA+ acetyl-CoA levels as well as enhanced vegetative and reproductive growth and salt/osmotic stress tolerance. In addition, dry seeds of overexpressing lines contained between 35–50% more fatty acids than did the wild type, suggesting that the CoA biosynthesis plays a crucial role in storage oil accumulation.

The action of *AtPPAT* in *Arabidopsis* suggests that overexpression of the *PPAT* gene also enhances metabolic activity and drought tolerance in jatropha; it is also expected to result in increased oil content. Therefore, we introduced the *AtPPAT* gene into jatropha (the Philippine line), which was designed to be constitutively overexpressed under the control of CaMV 35S promoter (pGWB11::*AtPPAT*). Transformed explants (Figure 2A) from cotyledons or young leaves were successfully regenerated, grew normally, and showed no phenotypic variation compared to wild-type plantlets (data not shown). The presence of the *AtPPAT* gene in the plantlets was examined by PCR. The DNA fragment of *AtPPAT* was detected in all the transformed plantlets, indicating that all contained the *AtPPAT* gene in their genomes (Figure 3A). In addition to the Philippine line used in previous studies (Khenkladngoen et al. 2011a, b), we also introduced the same construct into the Thai and Tanzanian lines of jatropha. Plantlets with roots were regenerated from explants of both lines (Figure 2B), and the expected fragments were amplified by PCR from genomic DNA samples of transformed explants (Figure 3B). Although the Thai line showed slightly better callus formation and shoot regeneration rate than others, the efficiency of other processes of the tissue culture was not significantly different from each other among the three lines.

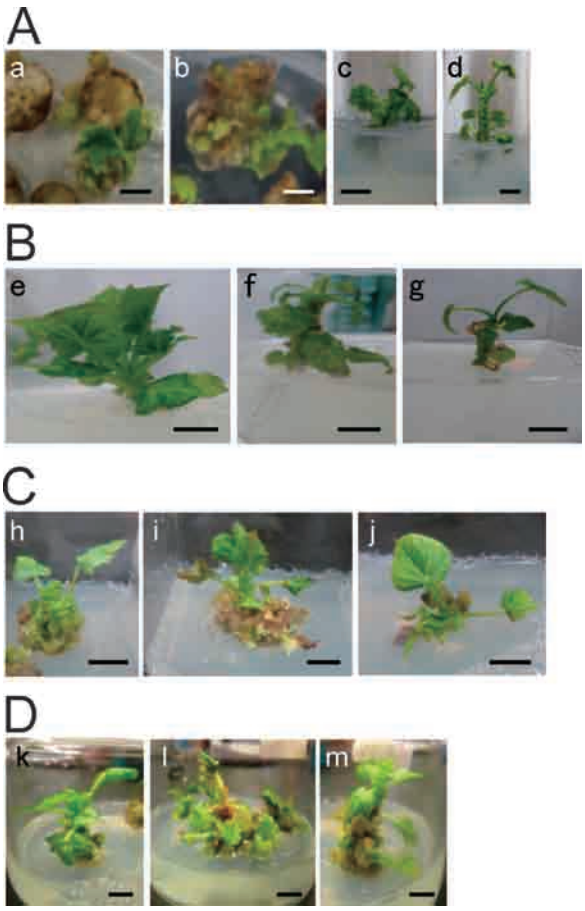

Figure 2. Regeneration of jatopha transformants. (A) Regeneration of *AtPPAT* transgenic plantlets obtained from the Philippine line. The early stage of regenerated shoots from cotyledonary explants (a) and leaf explants (b), as well as regenerated plantlets with induced roots (c, d). (B) Plant regeneration of *AtPPAT*-transformed explants obtained from the Thai (e, f) and Tanzanian (g) lines. Well-developed shoots in shoot elongation medium (e) and plantlets with roots (f, g). (C) Regenerated *AtNF-YB1* transformants in shoot elongation medium (h–j). (D) Regenerated *GSMT-DMT* transformants in shoot elongation medium (k–m). Bars: 1 cm.

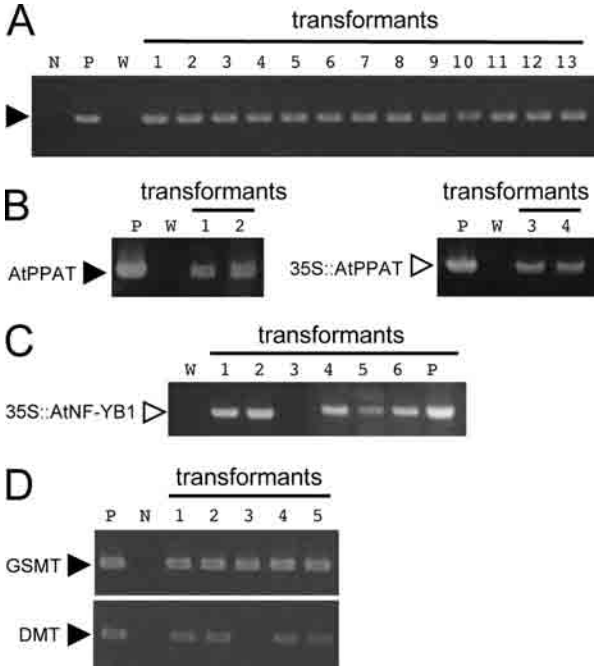

Figure 3. PCR analysis of transformants. (A) PCR analysis using a primer pair for *AtPPAT* (AtPPAT-F and AtPPAT-R). *Lanes*: N no template DNA, P pGWB11::*AtPPAT* plasmid, W DNA of a wild-type plantlet, 1–13 DNA of plantlets transformed by pGWB11::*AtPPAT* obtained from the Philippine line. (B) PCR analysis of *AtPPAT* transformed explants obtained from the Thai (left panel) and Tanzanian (right panel) lines. Primer pairs for *AtPPAT* (AtPPAT-F and AtPPAT-R) and CaMV 35S::*AtPPAT* (35S-F and AtPPAT-R) were used, respectively. *Lanes*: P pGWB11::*AtPPAT* plasmid, W DNA of a wild-type plantlet, 1 and 2 DNA of plantlets transformed by pGWB11::*AtPPAT* (the Thai line), 3 and 4 DNA of plantlets transformed by pGWB11::*AtPPAT* (the Tanzanian line). (C) PCR analysis using a primer pair for CaMV 35S::*AtNF-YB1* (35S-F and AtNFYB-R). *Lanes*: W DNA of a wild-type plantlet, 1–6 DNA of plantlets transformed by pGWB11::*AtNF-YB1*, P pGWB11::*AtNF-YB1* plasmid. DNA of 1, 3, and 5 were extracted from the plantlets h, i, and j in Figure 2C, respectively. (D) PCR analysis using a primer pair for *GSMT* (GSMT-F and GSMT-R) or that for *DMT* (DMT-F and DMT-R). *Lanes*: P pGWB11::*GSMT* or pGWB11::*DMT* plasmid, N no template DNA, 1, 2, 4 and 5 DNA of plantlets transformed with pGWB11::*GSMT-DMT*, 3 DNA of a plantlet transformed with pGWB11::*GSMT* and pGWB11::*DMT*. DNA of 1, 2, and 3 were extracted from the plantlets k, l, and m in Figure 2D, respectively.

Generation of NF-YB overexpressing plants

The *AtNF-YB1* gene of *Arabidopsis* encodes the B subunit of the nuclear factor Y (NF-Y complex, also known as the HAP or the CCAAT family), a conserved heterotrimeric complex consisting of the NF-YA (HAP2), NF-YB (HAP3), and NF-YC (HAP5) subunits, which mediates transcriptional control via CCAAT DNA elements (Mantovani 1999). This gene was identified as a putative regulator of drought-related stress tolerance in a large-scale functional genomics program performed in *Arabidopsis* (Nelson et al. 2007). *Arabidopsis* plants that constitutively express *AtNF-YB1* typically exhibit less severe wilting than do wild-type plants after a severe drought period, and show an increased rate of survival compared with the wild type upon re-watering following drought. During drought treatment, transgenic plants maintained higher water potential and photosynthesis rates than did the wild type, both of which are key phenotypes related to plant productivity. Interestingly, it is proposed that *AtNF-YB1* does not act via previously characterized drought-response mechanisms (Nelson et al. 2007).

The constitutive expression of a maize *AtNF-YB1* homolog in transgenic maize increases drought tolerance and enhances yield under drought stress (Nelson et al. 2007). To reinforce the productivity of jatropha in drought conditions, we introduced the *AtNF-YB1* gene, which is designed to be constitutively overexpressed under the control of CaMV 35S promoter (pGWB11::*AtNF-YB1*) into jatropha (the Thai line). Transgenic plantlets were regenerated on the medium

with Kan (Figure 2C); plantlets were also regenerated from non-transformed tissues on the medium without the drug. To determine the presence of the *AtNF-YB1* gene, PCR was performed to amplify the DNA fragment of *AtNF-YB1* from genomic DNA samples of six transformed plantlets as well as a non-transformed plantlet (Figure 3C). The DNA fragment was detected in five of six transformed plantlets, but not in the non-transformed plantlet, i.e., five of six transformed plantlets (1, 2, 4, 5 and 6) harboured the *AtNF-YB1* gene.

Generation of transgenic plants overproducing glycine betaine

In soils with limited amounts of available water, the salt concentration rises and consequently affects the plant growth. High salt concentration results in increased osmotic pressure causing osmotic imbalance in plant cells. Plant cells exposed to severe osmotic imbalance absorb water inefficiently. Some plant species with extremely high salt tolerance are able to protect themselves by producing a low molecular-weight compound called glycine betaine (reviewed by Khan et al. 2009). This compound works as an osmolyte that helps maintain high osmotic pressure in the cells, and thus enabling efficient water absorption even from saline water. Genetically engineered crops with enhanced glycine betaine biosynthesis show improved salt and drought tolerance; some of them also show improved tolerance to low temperatures (Khan et al. 2009). These findings indicate that these plants can adopt the strategy used by halophytes to survive in drought conditions via the protective nature of glycine betaine. Furthermore, the tolerance to low temperatures conferred by glycine betaine biosynthesis will be useful for growing crops in regions with lower temperature.

In plants, glycine betaine is formed by a two-step oxidation of choline. In the first step, choline is converted to betaine aldehyde, which is catalyzed by choline monooxygenase. In the second step, betaine aldehyde is converted to glycine betaine, which is catalyzed by betaine aldehyde dehydrogenase. Recently, the findings of a novel synthetic pathway to obtain glycine betaine were reported in halophilic phototrophic bacteria, including *Halorhodospira halochloris* (Nyyssola et al. 2000), *Aphanothece halophytica* (Waditee et al. 2003), and *Synechococcus* sp. WH8102 (Lu et al. 2006). In this pathway, glycine sarcosine methyltransferase (GSMT) catalyzes the methylation steps from glycine to sarcosine (*N*-monomethylglycine) and from sarcosine to dimethylglycine. Sarcosine dimethylglycine methyltransferase (DMT) catalyzes the steps from sarcosine to dimethylglycine and from dimethylglycine to glycine betaine. The co-overexpression of the *A. halophytica* GSMT and DMT in *Synechococcus* sp. PCC 7942 and *Arabidopsis* led to the accumulation of higher levels of betaine than previously reported for choline-oxidizing enzymes. The transgenic *Synechococcus* cells increased tolerance for salt stress. The transgenic *Arabidopsis* plants exhibited increased tolerance to salt, drought, and low-temperature stress, and produced significantly higher seed yields under the salt stress condition than that of the wild-type plants (Waditee et al. 2005).

In order to obtain jatropha lines with high yields under drought and other stress conditions, we generated transgenic jatropha (the Thai line) plants that co-expressed the *GSMT* and *DMT* genes of *Synechococcus* sp. WH8102 under the control of CaMV 35S promoter, and examined the accumulation of glycine betaine. The presence of the *GSMT* and *DMT* genes in transgenic plantlets was confirmed by PCR analysis. At first, we performed co-transformation of jatropha with plasmids containing the *GSMT* (pGWB11::*GSMT*) and *DMT* (pGWB11::*DMT*) genes. However, PCR analysis showed that it was difficult to obtain transformants that possessed both target genes together (see below). Therefore, we constructed and used a plasmid containing the two genes, pGWB11::*GSMT-DMT* (see Figure 1). All transformed plantlets were developed well in the regeneration medium as shown in Figure 2D. The PCR analysis of the resultant plantlets showed that four of five plantlets (1, 2, 4 and 5) transformed with pGWB11::*GSMT-DMT* harboured both of the *GSMT* and *DMT* genes, while one (3) transformed with pGWB11::*GSMT* and pGWB11::*DMT* harboured only the *GSMT* gene (Figure 3D).

The glycine betaine content of wild-type plants was determined by CE-MS analysis. Glycine betaine content ranged between 0.1 and 0.3 $nmol \cdot mgFW^{-1}$ in full-watered plants; this value did not increase after 2 weeks of drought treatment. These results suggest that the wild-type jatropha in this study might be the glycine betaine non-accumulator. In order to examine whether transgenic plantlets expressing *GSMT* and *DMT* accumulate glycine betaine, the glycine betaine content of two transgenic explants (1 and 2) as well as plantlets regenerated from non-transgenic tissues were determined. Glycine betaine content was about 0.2 $nmol \cdot mgFW^{-1}$ in the non-transgenic plantlets and higher (about 0.5 and 0.9 $nmol \cdot mgFW^{-1}$) in both plantlets transformed with *GSMT* and *DMT* (Figure 4). These results suggest that the expressions of the introduced *GSMT* and *DMT* genes significantly enhance glycine betaine synthesis in jatropha, and thus should effectively improve the drought tolerance of jatropha.

Improvement of the transformation protocol for generating transgenic jatropha plants

To develop a genetically modified plant through *Agrobacterium*-mediated transformation, one of the

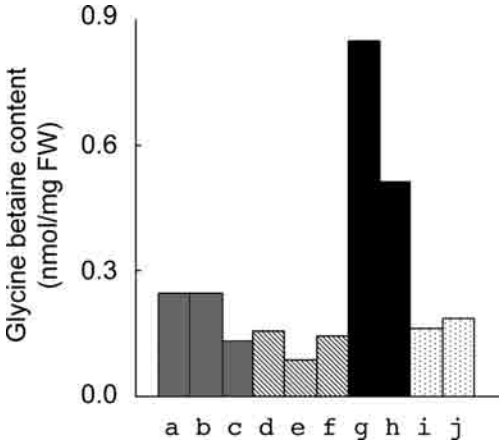

Figure 4. Glycine betaine content of jatropha plants. Bar graph indicating glycine betaine content of wild-type mature leaves of fully watered control plant (a–c), wild-type mature leaves exposed to 2 weeks drought stress (d–f), transformed plantlets (g and h), and wild-type plantlets (i and j) is shown. Samples of (g) and (h) in the graph were taken from plantlets (k) and (l) in Figure 2D, respectively.

crucial steps that must be dealt with is the regeneration of the transformed tissues into new plantlets. In this study, transformed explants were successfully regenerated using the regeneration system described by Khemkladngoen et al. (2011b) with some modifications for shoot regeneration and root induction. In addition, young leaves were used as plant materials. The use of young leaves is effective when the number of available seeds is limited because a single plant can provide many young leaves while a single seed can provide only two cotyledons. However, young leaves have less potential for plant regeneration ability than cotyledons. Therefore, the shoot regeneration medium for leaf explants was slightly modified. Since thidiazuron (TDZ) is useful for inducing a large number of shoot buds or shoots in jatropha (Deore and Johnson 2008; Khemkladngoen et al. 2011a), TDZ was added to the shoot regeneration medium (SR-I) to induce shoot buds in the first two weeks of culture. To eliminate the carry-over effect of TDZ, the leaf explants were transferred to the shoot regeneration medium without TDZ (SR-II) for further shoot multiplication and regeneration. The stepwise transfer of leaf explants is expected to facilitate efficient shoot regeneration from young leaves.

In this study, root induction from transgenic shoots was initially limited. Thus, improving root induction conditions was necessary. The presence of nutrient components has been shown to influence root production in many woody plants (Orlikowska 1992; Sriskandarajah et al. 1990). The presence of ammonium and nitrate in MS basal medium are thought to not only supply the nitrogen required for tissue growth, but also contribute to pH control in the growth medium. Ammonium causes acidity in the medium, while nitrate causes alkalinity. Previous studies demonstrated that combining quarter-strength MS macronutrients with full-strength MS micronutrients or media containing ammonium as the sole nitrogen source for rooting medium results in a gradual decrease in the pH of the medium during culture to 3.5–4 (Bennett et al. 2003; Woodward et al. 2006). Consequently, the availability of nutrients in the medium is limited, causing inefficient rooting of the shoots. Moreover, Bennett et al. (2003) revealed that the use of different nitrogen compounds other than NH_4NO_3 as nitrogen sources increases rooting efficiency in *Eucalyptus globulus*. Their results are consistent with our observation that root induction for the transgenic shoots was limited in half-strength MS basal medium (data not shown). Therefore, we used Gamborg's B5 medium, which contains KNO_3 as the major nitrogen source, and supplied lower NH_4^+ ($(NH_4)_2SO_4$) in the formulation (approximately 10 times lower than that in MS medium (NH_4NO_3)) as the root induction medium. The regenerated shoots were successfully rooted within 2–4 weeks of culture, and the number of roots per shoot (3–4 roots/shoot) was higher than that when MS medium was used (1–2 roots/shoot). Thus, the regeneration conditions described here are more efficient than those described in previous studies and can be applicable for efficient regeneration of transgenic plants from cotyledons and young leaves. Furthermore, it should be noted that our modified transformation system was applicable for three independent jatropha lines (from Philippines, Thailand, and Tanzania; see Figure 2), whereas other systems (Kumar et al. 2010; Li et al. 2008) are applicable only to the line that they specified.

Generation of the transgenic jatropha plants as well as the improved transformation system reported in this study would accelerate the development of new jatropha transgenic lines with high productivity in drought conditions. The diffusion of such lines may help jatropha cultivation spread without affecting the food production.

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