FIELD PERFORMANCE OF SELECTED MALAYSIAN COCOA CLONES REGENERATED THROUGH SOMATIC EMBRYOGENESIS CULTURES

GIBSON ENTUNI1*, REBICCA EDWARD1, HOLLENA NORI1 and AHMAD KAMIL MOHD. JAAFAR2

1Plant Science and Environmental Ecology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
2Malaysian Cocoa Board, Cocoa Research and Development Centre, Lot 248, Blok 14, Biotechnology Park, 94300, Kota Samarahan, Sarawak
*E-mail: gib5181@gmail.com

Accepted 26 January 2018, Published online 31 March 2018

ABSTRACT

Somatic embryogenesis is one of an efficient in vitro clonal propagation technologies with potential to be used to mass propagate cocoa clones in Malaysia. To ensure this technology for commercial production of cocoa across Malaysia can be applied, field performance of somatic embryogenesis-derived cocoa plants were evaluated for one year in Malaysian Cocoa Board, Kota Samarahan, Sarawak, Malaysia (MCB). Twenty-five cocoa plants derived from immature zygotic embryo and 25 cocoa plants derived from staminode explants of Trinitario variety were successfully propagated and acclimatized in greenhouse condition in UNIMAS before planted in field condition in MCB. Twenty-five mother plants from the same variety propagated through grafting were planted and used as control plants. At one year after planting, there were no major differences in growth parameters between somatic embryogenesis-derived plants with mother plants. Cocoa trees from immature zygotic embryo were slightly taller (998.1 mm), exhibit larger average stem diameters (34.6 mm) and taller jorquette branches (850.5 mm) than cocoa trees propagated through staminode cultures and grafting. After one year of field test, it can be concluded that somatic embryo-derived cocoa plants showed normal phenotypes and have growth parameters similar to cocoa plants propagated through conventional method of grafting.

Key words: Theobroma cacao, physiological characteristics, tissue culture, field experiment

INTRODUCTION

Theobroma cacao L., or simply known as cocoa tree is one of the most important cash crop trees currently grown in the humid tropics such as in Malaysia. In the last decade, its consumption has increased as it is cultivated for its fruit in which its seeds are used for the production of chocolates and confectionaries. Presently in Malaysia, cocoa trees are mainly propagated through seed as well as via rooting and grafting of plagiotropic cuttings. Cocoa is well known for its genetic variability due to its natural propagation system (allogamous), which generates a high degree of yield variation among the seed-derived plant (Maximova et al., 2002). The cocoa seeds are one of the main sources of heterozygosity of the crop in most cases, the result of cross linking between two genotypes. A large portion of low yielding trees in a single plantation was reported among the seed-derived plants (Irrizary & Rivera, 1999). In addition, there are also a number of disadvantages with the propagation of cocoa plants through rooting and grafting. These includes the need of intensive labor and thus costly, low propagation rate and formation of undesirable bush-like growth pattern of the cocoa plants. Although attempts have been made to develop organogenesis-based propagation methods, cocoa has demonstrated to be recalcitrant to in vitro shoot regeneration (Flynn et al., 1990).

Plant regeneration through somatic embryogenesis offers an alternative approach for clonal propagation of cocoa in Malaysia. Since somatic embryos are produced through bipolar development of somatic cells, plants derived from somatic embryos are morphologically identical to their mother plant...
as observed during the study on the field performance of somatic embryogenesis-derived cocoa plant in Saint Lucia, Brazil (Maximova et al., 2008) and Sumatera, Indonesia (Nurajijah et al., 2016). The study on the efficiency of the germination of somatic embryos and plantlet conversion of cocoa is still low and these barriers significantly affects the development of a successful somatic embryogenesis system in Malaysia (Tan, 2004). However, the previous related unpublished study has shown that plantlets conversion from somatic embryos of selected Malaysia cocoa clones has been successfully obtained. In cocoa, somatic embryogenesis is the most commonly adopted regeneration method, which has been used not only for plant propagation but also for genetic engineering (Loyola & Vasquez, 2006), virus elimination (Edward & Wetten, 2016) and germplasm preservation (Maximova et al., 2002).

Though, in a few specific cases, it was found that crops of other species produced through tissue culture showed high levels of genetic instability and somaclonal variation (Rival et al., 1997). In severe cases this propagation system resulted in sterile and very poor crop performance. For example, Matthes et al. (2001) found mutation in oil palm (Elaeis guineensis L.) such as flowers with mantled characters, parthenocarpic (seedless) fruit set and dwarfism. Kumar et al. (2011) also found mutations in banana (Musa L. sp.) including dwarfism, leaves with mosaic-like symptoms and reddish leaf petioles. In contrast, Vendrame and Faria (2011) reported little or no variation in crop plants such as in apple (Malus Mill. sp.), barley (Hordeum vulgare L.), cherry (Prunus avium x pseudocerasus L.) and pear (Pyrus communis L.). Irrespective of species, few data are available on the field performance of crop plants derived from somatic embryogenesis though such information is critical. Thus, before in vitro propagated cocoa are distributed on a wide scale in Malaysia, it is essential that field performance of the derived trees is carefully evaluated in field condition. Therefore, this study was conducted to evaluate somatic embryo-derived cocoa plant grown in the field for any beneficial and detrimental effects of the somatic embryogenesis process.

MATERIALS AND METHODS

Induction of somatic embryogenesis

The immature zygotic embryo and staminode explants were collected from mature grafted mother trees in MCB, Kota Samarahan, Sarawak that consistently produced high yield. Most of the selected trees were large and between six to seven years old. All of the trees are from the Trinitario heritage based on their morphological characteristics. The immature zygotic embryo and staminode explants were first surface sterilized before dissected and cultured onto plant tissue culture media for the induction of somatic embryos. The acclimated plants from Trinitario varieties were returned to Malaysian Cocoa Board Research Centre, Kota Samarahan Sarawak as bare rooted plantlets with the height between 10 cm to 12 cm. The plantlets were transplanted to 1:1 mixture of sand and soil in black plastic polybags and placed in the greenhouse with 50% netting shade under misting (Nurafiza et al., 2016) for 6 months prior to planting in the field. The grafted-derived cocoa clone from the same Trinitario heritage were also planted in black plastic polybags and grown as described above.

Field planting

In April 2016, a total of 75 cocoa trees were planted in the field in a completely randomized design with 3 m spacing enclosed with black plastic netting and shaded under established bamboo and banana plants. Growth data were collected for one year from April 2016 to April 2017. The growth parameters measured were stem diameter at 10 cm above the soil, height of the main stem from the soil to the first jorquette and the length of the longest jorquette branch. Means for the individual propagation methods and data collection points were calculated and variation was established by Fisher Protected LSD test at p<0.05 significance level. The times of flowering were also recorded for each of the individual trees. To test for the significant differences of these parameters among the propagation methods, Chi-square tests for homogeneity (p<0.05) was applied.

RESULTS AND DISCUSSION

After the regeneration under greenhouse condition in Universiti Malaysia Sarawak, bare root plants were transported to MCB, Kota Samarahan, Sarawak. The plants were then transplanted and acclimated in a greenhouse in plastic polybag with 100% survival rate. The plants were then transplanted to the field and interplanted with bamboo and banana plants (Figure 1a). Early survival rate was approximately 88% as 6 out of 50 trees derived from somatic embryo cultures derived cocoa plants died during the first 6 months of field planting due to drought condition. The cocoa plants were measured and photographed every months. The somatic embryogenesis culture-derived plants grew normally and showed dimorphic growth habit (Figure 1b). After one year of field planting, 16% of immature zygotic embryo-derived cocoa plants and 12% of staminode-derived cocoa plants had flowered (Figure 1c) and
produced fruits (Figure 1d). There was no significant difference in stem diameter between staminode and grafted control trees (p>0.47). The immature zygotic embryo-derived trees increasingly developed larger mean stem diameters compared to the staminode-derived trees and grafted control trees (Figure 2). The grafted control trees had significantly smaller stem diameter compared to somatic embryo culture-derived trees after one year of field planting (Table 1). Besides, no significant variation in stem diameter in correlation with different clones was found (data not shown).

The measurements of the main stem of each of the cocoa trees were evaluated every month from April 2016 to April 2017 from the soil surface to the shoot apex for young cocoa plants and as the trees had produced jorquette, from the soil to the first jorquette branch. It was found that all of the trees had achieved stem height between 850 mm to 994 mm after one year of field planting (Table 2). The normal development of cocoa trees and the transition from its juvenile to adult phases of development was best measured according to their stem height, stem diameter as well as the length of the jorquette branches (Maximova et al., 2008).

Minor increase on the average stem height was observed after 6 months of field planting. Comparison on stem height between somatic embryogenesis cultures-derived trees with the mother trees demonstrated that immature zygotic embryo-derived trees grew slightly taller than the other trees during one year of field planting (Figure 3). This followed by staminode cultures-derived trees with average stem height between 730 mm to 850 mm. The findings recorded during nursery stage also demonstrated that immature zygotic embryo-derived cocoa plant had the highest stem height compared to staminode-derived cocoa trees (Nurafiza et al., 2016). The grafted control mother trees were not significantly different from the staminode-derived trees (p>0.9) and immature zygotic embryo-derived trees (p>0.08) for the rest of the measuring period.

Another marker of cocoa trees development was the length of the longest jorquette branch. For field experiment, the cocoa plants with lower jorquettes
Fig. 2. Average stem diameter of cocoa trees propagated by somatic embryogenesis cultures and grafting.

Table 1. Mean stem diameters±SE (mm) of cocoa tree derived from somatic embryogenesis cultures and grafted control trees

<table>
<thead>
<tr>
<th>Type of cocoa tree</th>
<th>Mean stem diameter±SE (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staminode-derived tree</td>
<td>24.6±2.34a</td>
</tr>
<tr>
<td>Immature zygotic embryo-derived tree</td>
<td>34.6±2.40b</td>
</tr>
<tr>
<td>Grafted control tree</td>
<td>22.3±1.93a</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different at p>0.05 based on LSD test.

Table 2. Mean stem heights±SE (mm) of cocoa trees derived from somatic embryogenesis cultures and grafting control trees

<table>
<thead>
<tr>
<th>Type of cocoa tree</th>
<th>Mean stem height±SE (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staminode-derived tree</td>
<td>850.5±70.49a</td>
</tr>
<tr>
<td>Immature zygotic embryo-derived tree</td>
<td>994.4±38.60a</td>
</tr>
<tr>
<td>Grafted control tree</td>
<td>860.6±47.40a</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different at p>0.05 based on LSD test.

Fig. 3. Average stem height to the first jorquette of cocoa trees propagated via somatic embryogenesis and grafting.
were not pruned and therefore, the average jorquette heights reflected the difference. The length of the longest jorquette branch was recorded at one year after field planting at the time of the data collection (Figure 4). There was no significant difference in mean length of the longest jorquette branch recorded for all of the cocoa trees (p>0.34) (Table 3). The grafted control trees had shorter jorquette branch than somatic embryogenesis-derived trees (between 390 mm to 490 mm).

Another developmental process assessed was the flowering age of cocoa in the field. Here, the percentage of cocoa that had produced flower after one year was evaluated (Table 4). It was found that all the cocoa trees that produced flowers had developed jorquettes. By one year after planting, 16% of immature zygotic embryo-derived trees and 12% of staminode-derived cocoa trees had flowered. None of the grafted control mother trees had produced flower during this one year of field experiment. It was found that the somatic embryogenesis-derived trees produced flowers earlier than grafted control trees. Maximova et al. (2008) also reported an early flowering in somatic embryogenesis-derived cocoa trees. A mature cocoa tree must have a sufficient size and physiological

---

**Table 3.** Mean length of the longest jorquette branch±SE (mm) of cocoa trees derived from somatic embryogenesis cultures and grafted control trees after one year of field planting

<table>
<thead>
<tr>
<th>Type of cocoa tree</th>
<th>Mean length of the longest jorquette branch±SE (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staminode-derived tree</td>
<td>490.72±30.29a</td>
</tr>
<tr>
<td>Immature zygotic embryo-derived tree</td>
<td>850.46±70.04a</td>
</tr>
<tr>
<td>Grafted control tree</td>
<td>570.42±30.49a</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different at p>0.05 based on LSD test.

**Table 4.** Percentage of cocoa trees had flowered after one year of field planting

<table>
<thead>
<tr>
<th>Type of cocoa tree</th>
<th>Total number of plants</th>
<th>Percentage of plant produce flower (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staminode-derived tree</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>Immature zygotic embryo-derived tree</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>Grafted control tree</td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different at p>0.05 based on LSD test.

---

Fig. 4. Average length of the longest jorquette branch of cocoa trees propagated via somatic embryogenesis and grafting.
vigor to sustain the energy demand of flowering as well as for fruit and seed growth (Maximova et al., 2008). In younger cocoa trees, although they are flowering, they do not always support fruit development as fruits are often aborted at an early stage of their development. However, in this study, somatic embryogenesis-derived trees can sustain fruit growth as early as one year of their field planting and this indicates that somatic embryogenesis-derived cocoa trees had superior physiological vigor than cocoa trees propagated through traditional method of grafting. In contrast, Tan (2004) stated that it normally take around 2 years for the Malaysian cocoa clones to produce flowers after field planting.

CONCLUSION

This is a pilot study on field performance of selected Malaysian cocoa clones regenerated via somatic embryogenesis cultures. This finding after one year of field planting showed that somatic embryogenesis-derived cocoa trees did not differ significantly from cocoa trees propagated through conventional method of grafting. The early flowering age and the ability to sustain fruit growth during the first year of field planting showed that somatic embryogenesis-derived cocoa trees had superior physiological vigor than mother trees of grafting. It can be confirmed that this technology is significantly beneficial for the improvement of cocoa varieties in term of plant breeding and thus could be used as one of the main propagation method for large scale commercial deployment of cocoa in Malaysia. In the future, the individual data on the comparison of morphological characteristic of flowers and the fruits of the somatic embryogenesis cultures-derived cocoa trees and mother trees will be further evaluated.

ACKNOWLEDGEMENTS

The authors are grateful to the MCB Kota Samarahan Sarawak for the cocoa tree samples and Faculty of Resource Science and Technology UNIMAS for the facilities.

REFERENCES


