Comparing the Effect of Plant Growth Regulators on Callus and Somatic Embryogenesis Induction in Four Elite Theobroma cacao L. Genotypes

Modeste Kan Kouassi
Laboratoire Central de Biotecnologies (LCB), Centre National de Recherche Agronomique (CNRA), KM 17 Route de Dabou, 01 BP 1740 Abidjan 01, Côte d’Ivoire

Jane Kahia and Christophe N’guessan Kouame
Côte d’Ivoire Country Program, World Agroforestry Center (ICRAF), Cocody Mermoz, 08 BP 2823 Abidjan, Côte d’Ivoire

Mathias Gnion Tahí
Programme Cacao, Centre National de Recherche Agronomique (CNRA), Station de Recherche de Divo, BP 808 Divo, Côte d’Ivoire

Edmond Kouablan Koffi
Laboratoire Central de Biotecnologies (LCB), Centre National de Recherche Agronomique (CNRA), Côte d’Ivoire, KM 17 Route de Dabou, 01 BP 1740 Abidjan 01

Abstract. The effect of plant growth regulators on callus and somatic embryogenesis induction in four Cocoa (Theobroma cacao) genotypes was studied. Flower explants were harvested early in the morning and cultured on Driver and Kuniiyuki Walnut (DKW) medium supplemented with 1 mg·L⁻¹ of five auxins type (2,4 dichlorophenoxyacetic acid (2,4-D), 3,4 dichlorophenoxyacetic acid (3,4-D), 2,4,5 trichlorophenoxyacetic acid (2,4,5-T), 4-amino-3,5,6-trichloropicolinolic (piloram), and 3,6-dichloro-2-methoxybenzoic acid (dicamba) in combination with 0.25 or 0.5 mg·L⁻¹ of two cytokinins type (benzylaminopurine (BAP) and 6-furfurylaminopurine [kinetin (Kin)]) in a factorial experiment. The plant growth regulators 2,4-D and 2,4,5-T proved to have a broad spectrum action on somatic embryogenesis induction compared with 3,4-D or piloram. There were no significant differences between the two concentrations of cytokinins. However, Kin was found to be more effective in promoting somatic embryogenesis than BAP. Combining 1 mg·L⁻¹ 2,4,5-T or 2,4-D with 0.25 mg·L⁻¹ Kin had a broad spectrum action on embryogenesis induction. On the other hand, combining mg·L⁻¹ piloram with 0.5 mg·L⁻¹ Kin or 1 mg·L⁻¹ 3,4-D with 0.25 mg·L⁻¹ Kin was only able to induce somatic embryogenesis in a few of the genotypes evaluated. The protocol developed during the current study differs from earler works as the callus (derived from explants cultured on DKW media) was taken directly to embryo development media as opposed to earlier works in which the callus was taken through a secondary media before being transferred to an embryo development media.

Cocoa (T. cacao) is a neotropical, small, evergreen tree and native to the undergrowth of the Amazon forest (South America), and belongs to the Malvaceae family. It is cultivated around the world, for its seeds mainly used in the manufacture of chocolate and cosmetics. Côte d’Ivoire is the world’s leading cocoa producer accounting for more than a third of the global supply. Cocoa plays a key role in the economy of the country contributing to 15% of its GDP, 40% of its exports, and supporting more than 6 million people (Conseil Café Cacao, 2014; ICCO, 2012). Theobroma cacao is generally a heterozygous plant with a high variability for agronomic and quality traits. Seed propagation, while efficient, is problematic, since many of the trees are unproductive. The number of planting materials produced by rooted cuttings and grafting are unbalanced and far below the quantity needed to supply farmer’s demand. Therefore, there is a need to accelerate the production of the elite cocoa planting materials by using alternative methods such somatic embryogenesis. Somatic embryo production and plantlet regeneration have been achieved in a large number of genotypes (Li et al., 1998; Maximova et al., 2002).

Despite the current progress in this area, the reported efficiencies of somatic embryogenesis and plant regeneration obtained remain low. Furthermore, the practical utilization of this technology for clonal propagation remains hindered by an inability to induce somatic embryogenesis from a majority of elite cocoa genotypes. For these applications to be technically and economically feasible, it is essential to optimize the system variables to obtain high multiplication rates of quality somatic embryos. This is in view of the fact that different cocoa genotypes react differently to different callus-inducing hormones (Traore and Guilittian, 2006). Plant growth regulators play a key role by intervening in the reactions that lead to a reorientation of the program of gene expression. This expression can lead either to an unorganized growth of the cells (callus) without embryogenesis or to a polarized growth leading to a somatic embryogenesis (Dudit et al., 1995).

In T. cacao, Li et al. (1998) added 18 µM 2,4-D and 45.4 µM thidiazuron in DKW medium basal salts (Driver and Kuniiyuki, 1984) while López-Baez et al. (2000) used 2,4-D or 2,4,5-T (1 mg·L⁻¹) and Kin or 2 Isopentylenedine (2-ip) (0.25 mg·L⁻¹) in a modified Murashige and Skoog (MS) salts (Murashige and Skoog, 1962). These two systems resulted in somatic embryos production but some differences in efficiencies were apparent. The DKW basal salts media appears to be superior to MS media for cocoa tissue culture and although modifications of MS have given adequate results, most laboratories have switched to DKW based media (Guilittian and Maximova, 2000). Although the different combinations have been reported to enhance somatic embryogenesis efficacy, it remains evident that different clones vary in their response given to carbohydrate sources, plant growth regulators, or basal media (Guilittian and Maximova, 2000).

The present study was aimed at improving the induction of T. cacao somatic embryogenesis by combining several auxins and cytokinins. To the best of our knowledge, 3,4-D and dicamba have never been evaluated for induction of somatic embryogenesis of T. cacao.

Materials and Methods

Explants preparation. Flower buds about 4 to 5 mm long were collected (early in the morning before 9 am) in May 2014 from four elite genotypes coded C1, C9, C14, and C16 from Center National de Recherche Agronomique experimental farm in Divo (Côte d’Ivoire). The flower buds were surface-sterilized by using 1% (w/v) calcium hypochlorite for 20 min under the laminar flow cabinet. They were then rinsed four times in sterile distilled water and afterward sliced perpendicular to their longitudinal axis using...
Fig. 1. Steps, time duration, and type of media used to induce embryogenic calli in four cocoa genotypes. ED = embryo development; C.E = callogenic explants assessment; E.C = embryogenic calli assessment.

Table 1. Summary table for the percentage of callogenic explants (petal and staminode) of four cocoa genotypes on induction medium supplemented with auxins and cytokinins in a 4 × 4 factorial experiment.

| Source of variation | DF  | MS  | P value  | MS  | P value  |
|---------------------|-----|-----|----------|-----|----------|
| Clone               | 3   | 3.49| <0.001   | 17.04| <0.001   |
| Auxin               | 4   | 10.84| <0.001 | 17.00| <0.001   |
| Cytokinin           | 3   | 3.47| <0.001   | 1.22 | 0.003    |
| Clone × auxin       | 12  | 0.83| 0.131    | 0.42 | 0.008    |
| Clone × cytokinin   | 9   | 0.88| 0.123    | 2.29 | <0.001   |
| Auxin × cytokinin   | 12  | 2.14| <0.001   | 1.45 | <0.001   |
| Clone × auxin × cytokinin | 36 | 1.22| <0.001   | 0.96 | <0.001   |
| Residual            | 163 | 0.56| 0.26     |      |          |

DF = degrees of freedom; MS = mean square.

Table 2. Effects of auxins and genotypes on callogenic explant induction in cocoa. Observations were made after 4 weeks of culture.

| Factor | Levels of factors | Petals percentage of callogenic explants ± SE | Staminodes percentage of callogenic explants ± SE |
|--------|-------------------|---------------------------------------------|--------------------------------------------------|
| Auxin  | 2,4-D             | 88.67 ± 2.77 ab                             | 76.14 ± 3.54 ab                                 |
|        | 2,4,5-T           | 87.63 ± 2.94 ab                             | 81.38 ± 3.72 a                                  |
|        | 3,4-D             | 93.13 ± 2.88 a                              | 80.61 ± 3.68 a                                  |
|        | Picloram          | 62.10 ± 5.70 c                              | 45.97 ± 7.27 c                                  |
| Genotype| C1                | 85.93 ± 2.80 ab                             | 74.09 ± 3.57 ab                                 |
|        | C9                | 81.42 ± 2.57 ab                             | 38.71 ± 3.27 c                                  |
|        | C14               | 82.56 ± 3.17 ab                             | 76.85 ± 4.04 b                                  |
|        | C16               | 84.70 ± 3.22 ab                             | 85.91 ± 4.10 ab                                 |

2,4-D = 2,4 dichlorophenoxyacetic acid; 2,4,5-T = 2,4,5 trichlorophenoxyacetic acid; 3,4-D = 3,4 dichlorophenoxyacetic acid.

Table 3. Summary table for the percentage of embryogenic calli (from petal and staminode explants) of four cocoa genotypes on induction medium supplemented with auxins and cytokinins in a 4 × 5 × 4 factorial experiment.

| Source of variation | DF  | MS  | P value  | MS  | P value  |
|---------------------|-----|-----|----------|-----|----------|
| Genotype            | 3   | 4.79| <0.001   | 1.93| 0.04     |
| Auxin               | 4   | 5.85| <0.001   | 1.42| 0.02     |
| Cytokinin           | 3   | 5.01| <0.001   | 0.41| 0.67     |
| Genotype × auxin    | 12  | 2.02| <0.001   | 2.50| <0.001   |
| Genotype × cytokinin| 9   | 1.87| <0.001   | 1.33| 0.09     |
| Auxin × cytokinin   | 12  | 1.39| 0.007    | 0.97| 0.27     |
| Genotype × auxin × cytokinin | 36 | 1.03| 0.008    | 1.20| 0.04     |
| Residual            | 163 | 0.58| 0.79     |      |          |

DF = degrees of freedom; MS = mean square.

Results

Induction of callogenic explants. Depending on the explants, there was a slight difference in the effect of the factors auxin, cytokinin, and genotype on the induction of callus. With the petals, each factor and the interactions auxin × cytokinin and genotype × auxin × cytokinin had a high significant effect (P < 0.001) on the induction of callus. With staminodes, each factor and the interactions between the factors had a significant effect (Table 1).

Petals explants cultured on induction media supplemented with 3,4-D produced the highest percentage of explants with callus although it was not significantly different
The age of embryogenic calli varied according to the effect of the different factors on the percent-

zero response with both petals and staminodes are not indicated in the table.

Duncan test.

BAP = benzylaminopurine; Kin = kinetin.

The effects of combination auxin cytokinin on average of embryogenic calli induction from petals and staminodes in four cocoa genotypes.

From 2,4-D, 2,4,5-T, and picloram. The induction media supplemented with dicamba produced the lowest percent explants with calli. Petals were found to produce more calli than staminodes in all the genotypes evaluated. For the staminodes, C9 was found to form significantly higher percentage of calli than C1 (Table 2).

**Induction of somatic embryogenic calli.** The effect of the different factors on the percentage of embryogenic calli varied according to auxins evaluated. On the other hand, calli induced from staminodes formed the lowest percentage of embryogenic cultures. There were no embryo produced on media supplemented with dicamba from the two explants evaluated (Table 4). For each type of cytokinin, there were no significant differences between the two concentrations (0.25 or 0.5 mg L⁻¹) evaluated. Petal explants cultured on media supplemented with Kin produced significantly more embryogenic calli than those produced on BAP. On the other hand, staminodes produced lower embryogenic calii in media supplemented with both the cytokinin evaluated (Table 4).

Table 4. Effects of auxins, cytokinins, and genotypes on embryogenic calli induction in cocoa.

| Genotype | Auxin/cytokinin combination in induction medium | Avg percentage of embryogenic calli ± SE |
|----------|-----------------------------------------------|----------------------------------------|
|          | Petals                                        | Staminodes                              |
| C1       | 2,4-D/0.25 mg L⁻¹ BAP                         | 12.22 ce                                 |
|          | 2,4-D/0.25 mg L⁻¹ Kin                          | 22.59 bd                                 |
|          | 2,4-D/0.5 mg L⁻¹ BAP                           | 6.67 de                                  |
|          | 2,4-D/0.5 mg L⁻¹ Kin                           | 35.12 b                                  |
|          | 2,4,5-T/0.25 mg L⁻¹ BAP                        | 6.36 de                                  |
|          | 2,4,5-T/0.25 mg L⁻¹ Kin                        | 63.37 a                                  |
|          | 2,4,5-T/0.5 mg L⁻¹ BAP                          | 28.89 bc                                 |
|          | 3,4-D/0.25 mg L⁻¹ BAP                           | 5.00 ef                                  |
|          | 3,4-D/0.25 mg L⁻¹ Kin                           | 22.70 bc                                 |
|          | 3,4-D/0.5 mg L⁻¹ Kin                           | 10.82 ce                                 |
|          | Pic/0.25 mg L⁻¹ Kin                            | 6.67 de                                  |
|          | Pic/0.5 mg L⁻¹ BAP                             | 11.11 ab                                 |
| C14      | 2,4-D/0.25 mg L⁻¹ Kin                          | 2.22 ef                                  |
|          | 2,4-D/0.5 mg L⁻¹ Kin                           | 16.20 bc                                 |
|          | 2,4-D/0.5 mg L⁻¹ Kin                           | 7.41 de                                  |
|          | 3,4-D/0.25 mg L⁻¹ BAP                           | 4.76 ef                                  |
|          | 3,4-D/0.25 mg L⁻¹ Kin                           | 11.43 cd                                 |
|          | 2,4-D/0.25 mg L⁻¹ Kin                          | 22.02 bc                                 |
|          | 2,4-D/0.25 mg L⁻¹ Kin                          | 10.37 ce                                 |
|          | 2,4-D/0.5 mg L⁻¹ Kin                           | 32.12 b                                  |
|          | 2,4,5-T/0.25 mg L⁻¹ Kin                        | 36.67 b                                  |
|          | 2,4,5-T/0.5 mg L⁻¹ Kin                         | 6.67 de                                  |
|          | 3,4-D/0.25 mg L⁻¹ Kin                          | 36.67 b                                  |
|          | 3,4-D/0.5 mg L⁻¹ Kin                           | 10.37 ce                                 |
|          | 2,4-D/0.25 mg L⁻¹ BAP                           | 8.89 de                                  |
|          | 2,4-D/0.5 mg L⁻¹ BAP                           | 13.97 cd                                 |
|          | 2,4-D/0.5 mg L⁻¹ Kin                           | 11.43 cd                                 |
|          | 2,4,5-T/0.25 mg L⁻¹ BAP                        | 0 f                                     |
|          | 3,4-D/0.25 mg L⁻¹ BAP                           | 2.22 ef                                  |
| C9       | 2,4-D/0.25 mg L⁻¹ BAP                           | 10.37 ce                                 |
|          | 2,4-D/0.25 mg L⁻¹ Kin                          | 36.67 b                                  |
|          | 2,4-D/0.5 mg L⁻¹ Kin                           | 10.37 ce                                 |
|          | 2,4,5-T/0.25 mg L⁻¹ BAP                        | 0 f                                     |
|          | 3,4-D/0.25 mg L⁻¹ BAP                           | 11.75 cd                                 |
|          | 3,4-D/0.5 mg L⁻¹ BAP                           | 10.37 ce                                 |
|          | 3,4-D/0.25 mg L⁻¹ Kin                          | 6.67 de                                  |
|          | Pic/0.25 mg L⁻¹ BAP                            | 4.44 ef                                  |

BAP = benzylaminopurine; Kin = kinetin.

Mean values in each column followed by the same letter do not differ statistically at P < 0.05 according to Duncan test.

Zero response with both petals and staminodes are not included in the table.

2,4-D = 2,4 dichlorophenoxyacetic acid; 2,4,5-T = 2,4,5 trichlorophenoxyacetic acid; 3,4-D = 3,4 dichlorophenoxyacetic acid; BAP = benzylaminopurine; Kin = kinetin.

Values are mean ±SE. Mean values in each column followed by the same letter do not differ statistically at P < 0.05 according to Duncan’s test.

Table 5. Effects of combination auxin cytokinin on average of embryogenic calli induction from petals and staminodes.

| Genotype | Auxin/cytokinin combination in induction medium | Avg percentage of embryogenic calli ± SE |
|----------|-----------------------------------------------|----------------------------------------|
|          | Petals                                        | Staminodes                              |
| C1       | 2,4-D/0.25 mg L⁻¹ BAP                         | 12.06 ± 2.00 a                          |
|          | 2,4-D/0.5 mg L⁻¹ BAP                           | 5.14 ± 2.09 b                           |
|          | 2,4-D/0.5 mg L⁻¹ Kin                           | 0 d                                     |
| C9       | 2,4-D/0.25 mg L⁻¹ BAP                           | 4.65 ± 1.61 b                           |
|          | 2,4-D/0.5 mg L⁻¹ Kin                           | 3.40 ± 1.51 a                           |

2,4–D = 2,4 dichlorophenoxyacetic acid; 2,4,5–T = 2,4,5 trichlorophenoxyacetic acid; 3,4–D = 3,4 dichlorophenoxyacetic acid; BAP = benzylaminopurine; Kin = kinetin.

Dash significantly higher percentage of calli induction medium supplemented with dicamba from the two explants evaluated (Table 4).

For each type of cytokinin, there were no significant differences between the two concentrations (0.25 or 0.5 mg L⁻¹) evaluated. Petal explants cultured on media supplemented with Kin produced significantly more embryogenic calli than those produced on BAP. On the other hand, staminodes produced lower embryogenic calli in media supplemented with both the cytokinin evaluated (Table 4).

Petals were found to be the best explants for induction of somatic embryogenesis in C1, C14, and C16. In C9, there was no significant difference between the petals and staminodes. Genotype C1 exhibited the best mean percent of somatic embryogenesis (Table 4).

Auxins 2,4-D and 2,4,5-T combined with Kin were found to favor the induction of somatic embryogenesis in all genotypes. The petal explants produced the highest embryogenic response for all the genotypes when cultured on media supplemented with the combination of 1 mg L⁻¹ 2,4-D, 2,4,5-T/0.25 mg L⁻¹ Kin and the second best combination was 1 mg L⁻¹ 2,4-D/0.5 mg L⁻¹ Kin (Table 5). It was also observed that picloram and 3,4-D were found to be specific in that they were able to induce somatic embryogenesis in C16 and C9, respectively. Figure 2 shows somatic embryos of genotype C16 induced on medium supplemented with 1 mg L⁻¹ picloram and 0.5 mg L⁻¹ Kin.

**Discussion**

It was observed that 2,4-D and 2,4,5-T had a broad spectrum action on somatic embryogenesis compared with 3,4-D or picloram. The results of the current study are in line with those of López-Baez et al. (2000) who reported that the combinations of 0.25 mg L⁻¹ 2,4-D or 2,4,5-T led to a successful induction somatic embryogenesis in two genotypes (H20 and H31). In the present study, somatic embryos were induced on calli derived from petals with 1 mg L⁻¹ picloram. These results are contrary to the work of Zuyasna et al. (2012) who reported that the low concentrations (less than 3 mg L⁻¹) of picloram were not able to induce somatic embryogenesis in north ace cocoa genotype. A possible explanation could be due to the differences in the genetic make up. It was also observed that the petals produced higher embryogenic cultures than staminodes explants in all the genotypes evaluated. These results are in contrast with those of Zuyasna et al. (2012) who found that staminode explants produced 3 to 10 times more somatic embryos than petals. This could be because the cacao genotypes used by Zuyasna et al. (2012) were different from those evaluated in the current study. Indeed genotype dependent response would need to be considered.
of cacao somatic embryogenesis has been reported previously (Ajijah et al., 2014).

The performance of 3,4 D in C9 was comparable with 2,4-D which is the most used auxin in cocoa somatic embryogenesis. In rubber (Hevea brasiliensis), 3,4-D which is considered as a “weak” auxin was found to give better result compared with 2,4-D when combined with BAP (Michaux-Ferrière and Carron, 1989). An attempt to induce somatic embryogenesis with calli obtained from media supplemented with dicamba was not successful. This may be explained by an inappropriate concentration of auxin (dicamba) or its combination with an inadequate or a suboptimum concentration of cytokinin (Kouassi et al., 2013). In oil palm, the highest haustorium embryos producing embryogenic callus was obtained from primary callus after culture on MS medium supplemented with 1 mg L⁻¹ dicamba in the presence of 200 mg L⁻¹ ascorbic acid (Chehmalee and Te-chato, 2008). In the current study, no embryo was produced on induction medium supplemented with dicamba. This is the first report of the use of dicamba and 3,4-D to evaluate callus and somatic embryogenesis induction in cocoa.

In this study, for each type of cytokinin, there were no significant differences between the two concentrations evaluated. It is therefore recommended to use the lowest concentration to induce somatic embryogenesis. Petals were found to be the best explants for induction of somatic embryogenesis in all the genotypes evaluated except in C9 in which both petals and staminodes had similar embryogenic response. The results of the current study concur with those of Ajijah et al. (2016) have reported that Kin in combination with 2,4-D induced higher responses of somatic embryogenesis when basal petals were used compared with staminodes in some genotypes (GC7, ICC212, and Cinmandu2). On the contrary, in other genotypes (Sca 6, ICS 13, and DR 2) staminode explants showed better responses than petals. In the present study, it was also observed that for responsive genotypes, induction media supplemented with Kin were more embryogenic than that with BAP. However, for less responsive genotype, Kin could have the same effect as BAP.

**Conclusion**

Auxins 2,4,5-T and 2,4-D in combination with Kin were observed to have a broad spectrum action on somatic embryogenesis induction. It was also demonstrated for the first time ever that it is possible to use picloram and 3,4-D to induce callus and somatic embryogenesis respectively in the elite cocoa genotypes. However, it is necessary to evaluate the quality of somatic embryos regenerated using the two cytokinins.

**Literature Cited**

Ajijah, N., R.S. Hartati, R. Rubiyo, D. Sukma, and S. Sudarsono. 2016. Effective cacao somatic embryo regeneration on kinetin supplemented DKW medium and somaclonal variation assessment using SSRS markers. Agrivita 38(1):80-92.

Ajijah, N., Rubiyo, and Sudarsono. 2014. Calllogenesis and somatic embryogenesis of cacao using thidiazuron through one step of callus induction (in Indonesian). J. Penelitian Pertanian Tani Man Industri 20(4):179-186. <http://www.ejournal.litbang.pertanian.go.id/index.php/jptip/article/view/2580/2220>.

Chehmalee, S. and S. Te-chato. 2008. Induction of somatic embryogenesis and plantlet regeneration from cultured zygotic embryo of oil palm. J. Agr. Tech. 4(2):137-146.

Conseil Café Cacao. 2014. Programme Qualité-Qualité-Croissance « 2QC » 2014-2023. Le Conseil de Régulation, de Stabilisation et de Développement de la filière café-cacao. Dec. 2016. <www.conseilcafecacao.ci/docs/Programme_2QC_2014-2023.pdf>.

Driver, J.A. and A.H. Kuniyuki. 1984. In vitro propagation of Paradox walnut rootstock. HortScience 19:507–509.

Dudits, D., J. Görgényy, L. Bogre, and L. Bakó. 1995. Molecular biology of somatic embryogenesis, p. 267–308. In: T. A. Thorpe (ed.) In Vitro Embryogenesis in Plants. Kluwer Academic Publishers, Dordrecht.

Guiltinan, M.J. and S. Maximova. 2000. Recent advances in the tissue culture of cocoa from somatic embryos to bentwood gardens: A short review. In Abstr. Ingenic Workshop on the New Technologies and Cocoa Breeding. Kota Kinabalu, Malaysia.

International Cocoa Organization (ICCO). 2012. How many smallholders are there worldwide producing cocoa? What proportion of cocoa worldwide is produced by smallholders? Aug. 2016. <http://www.icco.org/faq/planta-cultural/123-how-many-smallholders-are-there-worldwide-producing-cocoa-what-proportion-of-cocoa-worldwide-is-produced-by-smallholders.html>.

Kouassi, K.M., K.E. Koffi, N.G. Konkon, M. Gnagne, M. Koné, and T.H. Kouakou. 2013. Influence of plant growth regulators on somatic embryogenesis induction from inner teguments of rubber (Hevea brasiliensis) seeds. Afr. J. Biotechnol. 12(16):1972–1977.

Li, Z., A. Traoré, S.N. Maximova, and M.J. Guiltinan. 1998. Somatic embryogenesis and plant regeneration from floral explants of cocoa (Theobroma cacao L.) using Thidiazuron. In Vitro Cell. Dev. Biol. 34:293–299.

López-Baez, O., J.L. Moreno-Martínez, and S. Pacheco-Rodas. 2000. Advances in cocoa Theobroma cacao propagation by somatic embryogenesis in Mexico. In Abstr. 3rd Ingenic Workshop on the New Technologies and Cocoa Breeding. Conseil de Régulation, de Stabilisation et de Développement de la filière café-cacao. Dec. 2010. Programme Quantité-Qualité-Croissance « 2QC » 2014-2023. Le Conseil de Régulation, de Stabilisation et de Développement de la filière café-cacao. <http://www.conseilcafecacao.ci/docs/Programme_2QC_2014-2023.pdf>.

Maximova, S.N., L. Alemano, A. Youg, A. Traoré, N. Ferrier, and M.J. Guiltinan. 2002. Genotype variability, efficiency and cellular origin of primary and secondary somatic embryogenesis of Theobroma cacao L.: the chocolate tree. In Vitro Cell. Dev. Biol. 38:252–259.

Michaux-Ferrière, N. and M.P. Carron. 1989. Histology of early somatic embryogenesis in Hevea brasiliensis. The importance of timing of subculturing. Plant Cell Tissue Organ Cult. 19:243–256.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–497.

Traoré, A. and M. Guiltinan. 2006. Effects of carbon source and explants type on somatic embryogenesis of four cocoa genotypes. Hort-Science 41:753–758.

Zuyasna, S. Hafisha, R. Fajri, O.M. Syahputra, and G. Ramadhan. 2012. The effect of picloram concentrations and explants types on the induction of somatic embryo on North Aceh Cocoa genotype. Poster, Proc. 2nd Annu. Int. Conf. Syiah Kuala Univ. 2012 and The 8th IMT-GT Uninet Biosc. Conf. Band Aceh, Vol. 2 Number 1.