Somatic embryogensis in native cacao from Amazonas using Thidiazuron

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BACKGROUND

Different researchers have discussed the embryogenesis in cacao, from Alemanno et al. (1996), to Henao et al. (2018). Contributions such as those of Li et al. (1998); Tan and Furtek (2003); Maximova et al. (2005) have contributed to the development of efficient *in vitro* culture protocols for cacao. Subsequently Trujillo et al. (2011) and Henao et al. (2018) have demonstrated the benefits and capabilities of somatic embryogenesis. However, there are still different responses among genotypes and culture protocols.

The aim of this study was to evaluate the embryogenic response of native cacao from Amazonas using thidiazorun (TDZ) and to initiate the establishment of protocols for the reproduction of native cacao clones by somatic embryogenesis.

METHODS

A trial was set up in CRD with a 10A (genotypes) x 3B (TDZ concentrations) factorial arrangement. The culture media and protocols used in this study were developed by Maximova et al. (2005).

Collection: Closed floral buds were collected from the field and stored at 1-16°C. for a maximum of 3 days.

Sterilization: Floral buds were immersed in sodium hypochlorite (2% active chlorine) for 20 min and washed three times with sterile water.

Culture: Floral buds were dissected to recover the staminodes. Staminodes were cultured on PCG medium (25 per dish) for 14 days at 26°C in the dark.

Subcultures: The first subculture was in SCG medium for 14 days at 26°C. Subsequent subcultures in ED medium. Refresh every 14 days. Maintained at 26°C in the dark.

THESE STEPS WERE PERFORMED IN A LAMINAR FLOW CABINET

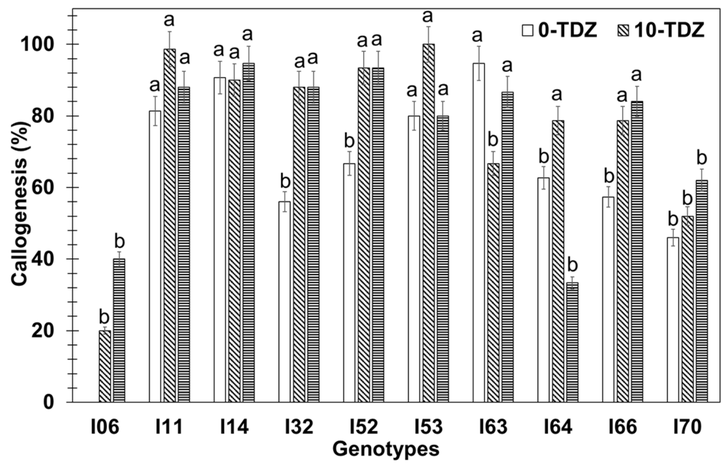
To determine the effect of the treatments, the percentage of callogenesis, embryogenesis and the number of embryos were measured.

Table 1: Genotypes used in the study

Code	UTM	Zone
Indes-06	9369168 17M 787894	El Chalán
Indes-11	9369112 17M 787792	El Chalán
Indes-14	9366961 17M 793728	El Limoncito
Indes-32	9367833 17M 779564	Quebrada Seca
Indes-52	9366649 17M 794441	Diamante Bajo
Indes-53	9366665 17M 794453	Diamante Bajo
Indes-63	9365734 17M 793806	Naranjos Alto
Indes-64	9364133 17M 792251	Naranjos Alto
Indes-66	9364181 17M 792346	Naranjos Alto
Indes-70	9371938 17M 787756	Lluhuana

RESULTS

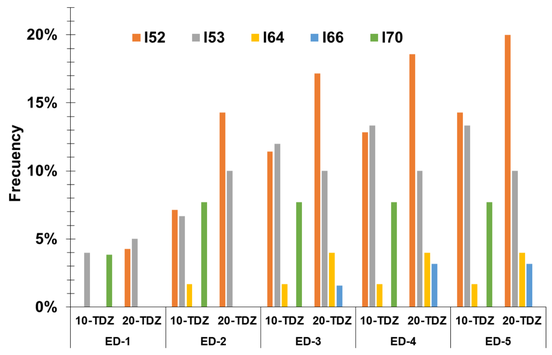
Figure 1: Percentage of callogenesis at 28 days of culture.



At 28 days of culture, all genotypes formed callus on at least one treatment.

Most TDZ-containing treatments achieved higher levels of callogenesis.

Figure 2: Percentage of embriogenic explants.



At the end of the culture period (ED 5) only 5 genotypes in 8 treatments were able to form primary somatic embryos.

Table 2: Embryos number per embriogenic treatments.

Genotype	[TDZ]	ED5 (70 days) Embriogenic Explants	Embyos per explant	Maximum Embryos per explant	Total embryos
Indes-52	10-TDZ	10	**17.6**	**44**	176
Indes-52	20-TDZ	**14**	16.93	39	**237**
Indes-53	10-TDZ	10	5.1	10	51
Indes-53	20-TDZ	4	15.5	38	62
Indes-64	10-TDZ	1	1	1	1
Indes-64	20-TDZ	1	22	22	22
Indes-66	20-TDZ	2	3.5	4	7
Indes-70	10-TDZ	2	28	32	56

The INDES-52 genotype achieved a high number of total embryos, although the embryogenic frequency was low in this study.

Figure 3: Callus in native cacao staminodes.

Figure shows different appearance positions of callus.
A: Callus at the base of the staminode.
B: Callus in the middle part of the staminode.
C: Callus in the distal part of the staminode.
D: Callus on the basal and middle parts of the staminode.
E: Callus on the basal and distal parts of the staminode.
F: Callus on the entire staminode.

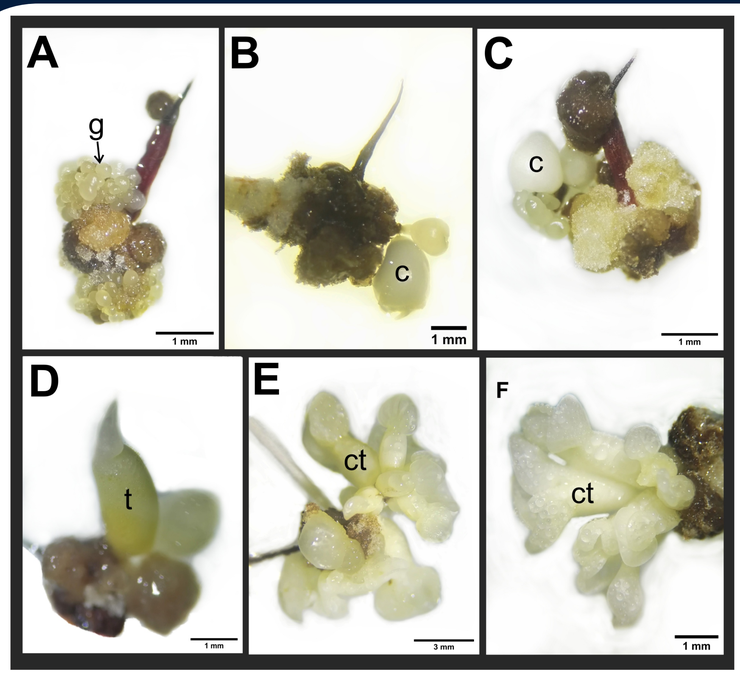


Figure 4: Cocoa somatic embryogenic stages.

Figure shows different development stages of embryos during ED culture phase.

Globular (g).

Heart (c).

Torpedo (t).

Cotyledonary (ct).

CONCLUSION

The generation of somatic embryos was achieved in five native cacao genotypes of Amazonas. This represents a great potential for works that attempt to improving the scale of multiplication or intend to use this tool as a way for genetic improvement and massive multiplication of cacao in Amazonas.This will make it possible to offer cocoa farmers in the region good quality cocoa seeds, solving the problem of the shortage of superior seeds.

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