Chapter 14

Somatic embryo germination of *Psidium guajava* L. in the Rita® temporary immersion system and on semisolid medium

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Abstract: Germination of somatic embryos and development to plants is recognized as one of the most critical stages in the process of plant propagation via somatic embryogenesis. The objective of the study was to investigate the germination of somatic embryos of Psidium guajava cv. Cuban Red Dwarf EEA 18-40 in the RITA® system and on semisolid medium. Somatic embryos were obtained from immature zygotic embryos which were cultured on the major salts of MS medium at half strength, supplemented with 400 mg l^{-1} L-glutamine, 100 mg l^{-1} ascorbic acid, 60 g l^{-1} sucrose and 1 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D). Somatic embryos at the heart and torpedo stages were transferred for germination into RITA® vessels containing liquid half strength MS medium of the major salts supplemented with 0.25 mg l^{-1} 6- benzylaminopurine (6-BAP), 10 µg l^{-1} Biobras-6 (brassinosteroid analogue) and 20 g l^{-1} sucrose or to semiliquid medium of the same composition (solidified with 2.5 g l^{-1} Gellum Gum, Spectrum®) in 250 ml glass vessels. The germination percentage, fresh weight and number of somatic embryos with complete germination were determined. After 10 weeks of culture the highest germination percentage (91%) and fresh weight (1.22 g) were achieved in the temporary immersion system, being statistically superior to those obtained from semisolid culture medium (81.79% and 1.03 g respectively).

Key words: Guava, liquid medium, somatic embryogenesis

1. Introduction

Among 150 *Psidium* species guava (*Psidium guajava* L.) presents the greatest potential from an economic point of view. Difficulties in the

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application of conventional propagation techniques have generated considerations of other possible forms of vegetative propagation (Pontikis, 1996). Especially somatic embryogenesis, which has been investigated as a tool for genetic improvement and for the propagation of elite trees (Vilchez, 2001).

The germination of somatic embryos has proved to be one of the most critical stages in somatic embryogenesis (Merkle et al., 1995). Temporary Immersion Systems (TIS) have been successfully used in several protocols for the germination of somatic embryos of some species (Etienne-Barry et al., 1999; Gómez et al., 2000). In this research, germination of guava somatic embryos in TIS was studied and compared to cultures on semisolid medium.

2. Materials and methods

Somatic embryos of *Psidium guajava* L. cv. 'Cuban Red Dwarf EEA 18-40', were obtained from immature zygotic embryos at the torpedo and cotyledonary stages. These were cultured on the major salts of MS medium (Murashige and Skoog, 1962) at half strength, supplemented with 400 mg l^{-1} L-glutamine, 100 mg l^{-1} ascorbic acid, 60 g l^{-1} sucrose and 1 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D). Subculturing was performed at three-week intervals using the same culture medium.

The operations and characteristics of the RITA®system (CIRAD, France) have been described by Etienne-Barry et al., (1999). For embryo germination each RITA® unit contained 200 ml of liquid MS culture medium with 50% of the major salts supplemented with 0.25 mg l^{-1} 6-benzylaminopurine (6-BAP), 10 µg l^{-1} Biobras-6 (brassinosteriod analogue from Nature Product Lab., Havana University) and 20 g l^{-1} sucrose. The inoculum density per RITA vessel was 800 mg fresh weight of somatic embryos at the heart or torpedo stages. The immersion frequency was one minute every 12 hours. The experiments were duplicated.

For control cultures 250 ml glass vessels were used with 30 ml of semisolid culture medium (solidified with 2.5 g l^{-1} Gellan gum, Spectrum®) consisting of the same composition as used for the RITA® units. Ten culture vessels were inoculated with the 800 mg fresh weight of somatic embryos as described for RITA® cultures.

The experiment was carried out in a growth chamber with the natural photoperiod in Cuba according to the time of the year (November-January) and a photosynthetic photon flow superior to 60 µmol m^{-2} s^{-1}. The temperature regime was 25 ± 2.0 ºC. After ten weeks of culture, the percentage of germinated embryos and fresh weight were determined. The

germination percentage was analyzed statistically through the proportion comparison test, complemented with Fisher's exact test and the fresh weight was compared by analysis of simple variance and the Tukey multiple range test.

3. Results and discussion

Both in the TIS and in semisolid culture medium, germination of the somatic embryos started around ten days after placement in the germination medium, when the embryo changed color from white to a shiny green. Table 1 shows the comparison between the germination of the somatic embryos in TIS and in semisolid culture medium. The statistical analysis points out significant differences for the variables germination percentage and fresh weight.

Table 1: Comparison of the germination percentages and fresh weight of the somatic embryos of *Psidium guajava* L. developed in TIS and on semisolid culture medium

	Germination percentage*	Fresh weight (g)**
TIS	91.04 a	1.22 ± 0.15 a
Semisolid	81.79 b	1.03 ± 0.09 b

*Different letters differ statistically for $p<0.05$ according to the proportion comparison test complemented with Fisher's exact test.
**Different letters differ statistically for $p<0.05$ according to Tukey multiple range test.

The highest germination percentage (91 %) and fresh weight were obtained with embryos in the TIS, being superior to the results reported in a similar system by Etienne-Barry et al. (1999) for *Coffea arabica* (60%). The somatic embryos germinated in the TIS were morphologically similar to those germinated in semisolid culture medium (Figure 1A and B). Partial germination with shoot development in some somatic embryos was found in semisolid medium only.

Hyperhydricity was not observed (Figure 1C), an aspect also pointed out by Cabasson et al. (1997) in embryos of *Citrus deliciosa* and Escalona (1999) in shoots of *Ananas comosus* multiplied in TIS.

The differences between the germination of the somatic embryos in TIS and semisolid culture medium may be explained by the limited contact between the somatic embryos and the culture medium in the TIS, which is reduced to a thin layer of culture medium that adheres to the somatic embryos and is renewed during every immersion. This layer is too thin to inhibit gaseous exchange. The aeration is also better since the atmosphere is exchanged during medium transfer. Additionally, medium exchange causes agitation of the plant material (Teisson and Alvard, 1995).

Germination of somatic embryos of several species (Citrus, Musa and Coffee) that was not possible in agitated Erlenmeyer flasks or on semisolid medium has been obtained in the TIS (Teisson and Alvard, 1995; Cabasson et al., 1997). This does not occur in the semisolid culture medium, where the contact with the culture medium is permament and the gaseous atmosphere influences the process of differentiation, due to low concentrations of oxygen which obviously reduce the number of somatic embryos per explant.

4. Conclusion

With the use of the temporary immersion systems it is possible to achieve a more efficient germination of the somatic embryos of *Psidium guajava* cv. Cuban Red Dwarf EEA 18-40. Developing plants show normal morphological characteristics without features of hyperhydricity.

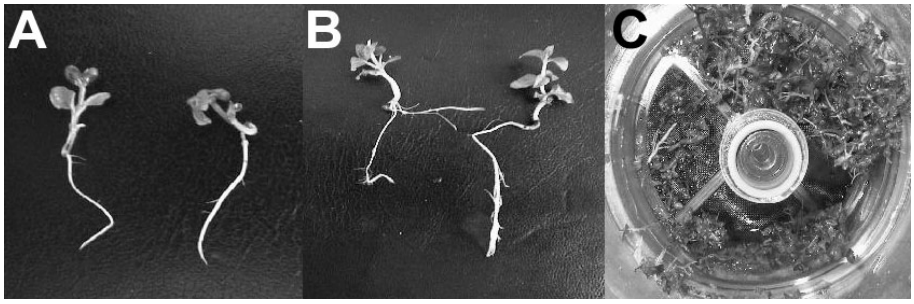

Figure 1: Morphology of germinated somatic embryos of *Psidium guajava* L. A: Embryos from RITA®. B: Embryos from semisolid culture medium. C: RITA® vessel with germinated embryos.

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