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NOTA CORTA / SHORT NOTE

BRASSINOSTEROIDS PREVENT THE CYTOKININ-INDUCED SOMATIC EMBRYOGENESIS OF COFFEE (*Coffea canephora*)

Los brasinoesteroides impiden la embriogénesis somática inducida por citocininas en el café (*Coffea canephora*)

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ABSTRACT

Plant somatic embryogenesis is a natural process that can be replicated *in vitro* by adding plant growth regulators. Reports indicate that the application of brassinosteroids increased the number of embryos when added to the cytokinin-induced somatic embryogenesis of *Coffea arabica*. Because somatic embryogenesis in the related *C. canephora* species is induced by cytokinins, we investigated whether brassinosteroids can also improve the embryogenic response in *C. canephora*. The results showed that the addition of 22(S),23(S)-homobrassinolide to *C. canephora* embryogenic explants prevented the establishment of the process in a dose-dependent manner, repressed the transcription of the *SERK1* gene homolog and increased the conductivity and acidification of the culture medium. The opposite effects of brassinosteroids on somatic embryogenesis of *C. canephora* and *C. arabica* demonstrate that the function of plant growth regulators during *in vitro* somatic embryogenesis is not conserved, even within species of the same genus.

Keywords: Cell differentiation, molecular regulation, plant tissue culture, plant growth regulators.

RESUMEN

La embriogénesis somática vegetal es un proceso natural que puede replicarse *in vitro* mediante la adición externa de reguladores del crecimiento vegetal. Existen reportes indicando que la aplicación de brasinosteroides incrementó el número de embriones cuando se adicionan a la embriogénesis somática inducida por citocininas en *Coffea arabica*. Debido a que la embriogénesis somática en *C. canephora*, una especie del mismo género es inducida por citocininas, se investigó si los brasinoesteroides pueden también mejorar la respuesta embriogénica en *C. canephora*. Los resultados mostraron que la adición de 22(S),23(S)-homobrasinólido a explantes embriogénicos de *C. canephora* impidió el establecimiento del proceso en una manera dependiente de la dosis, reprimió la transcripción del homólogo del gen *SERK1* y aumentó la conductividad y acidificación. del medio de cultivo. Los efectos opuestos de los brasinoesteroides sobre la embriogénesis somática de *C. canephora* y *C. arabica* demuestran que la función de los reguladores del crecimiento vegetal durante la embriogénesis somática *in vitro* no está totalmente conservada, incluso dentro de especies del mismo género.

Palabras clave: Cultivo de tejidos vegetales, diferenciación celular, regulación molecular, reguladores del crecimiento vegetal.

INTRODUCTION

Plant somatic embryogenesis (SE) is a natural process occurring in a few species, including those of the genus Kalanchoë (Kahraman *et al*. 2022), Bryophyllum (Du *et al*. 2023), and Malaxis (Taylor 1967). Development of *in vitro* SE has been achieved in many species by the addition of plant growth regulators (PGR), mainly auxins and cytokinins. In some species, BR is also involved in the acquisition of embryogenic competence, especially in *C. arabica*, where the addition of BR to the cytokinin-induced SE process increased the number of embryos and improved their morphology, suggesting that BR could be used to optimize the embryo regeneration system of *C. arabica* (Chone *et al*. 2018). In our group, we are studying the SE process of *C. canephora* (employing the Robusta variety), the commercially second important species of the genus *Coffea*. Our SE process is highly efficient, and it is also induced by cytokinins. Optimization of *in vitro* plant regeneration systems can help to increase commercial production of plants with desirable agronomic traits and to obtain a greater number of regenerants when plant clones are transformed for biotechnological purposes; thus, the objective of this work was to evaluate whether the addition of BR could further improve the SE process of *C. canephora*, as it does in *C. arabica*.

The SE process of *C. canephora* was triggered by the addition of 5 µM benzyladenine (BA) to 0.5 cm diameter disc leaf explants extracted from *in vitro* plants preconditioned for two weeks with 0.54 µM naphthaleneacetic acid and 2.32 µM kinetin, as described by Pérez-Pascual et al. (2018). Under these conditions, somatic embryos developed at the edges of the disc explants, with pro-embryogenic mass (PEM), globular embryos, and cotyledonal embryos developing between ten and 15, 15 and 20, and from 45 days after embryogenic induction, (dai), respectively (Fig. 1, the row labeled BA+BR-). Mature embryos had a 100 % conversion rate when transferred to the germination medium (Fig. 1b). Addition of different concentrations of the 22(S),23(S)-homobrassinolide (0.005, 0.05, and 0.5 µM) at the beginning of the SE process caused a dramatic decrease in the number of embryos, at all stages, in a dose-dependent manner (Fig. 1, rows labeled BA+BR+); embryos at the cotyledonal stage were reduced to 50±10 with 0.005 µM and almost abolished with 0.5 µM BR. In the absence of the

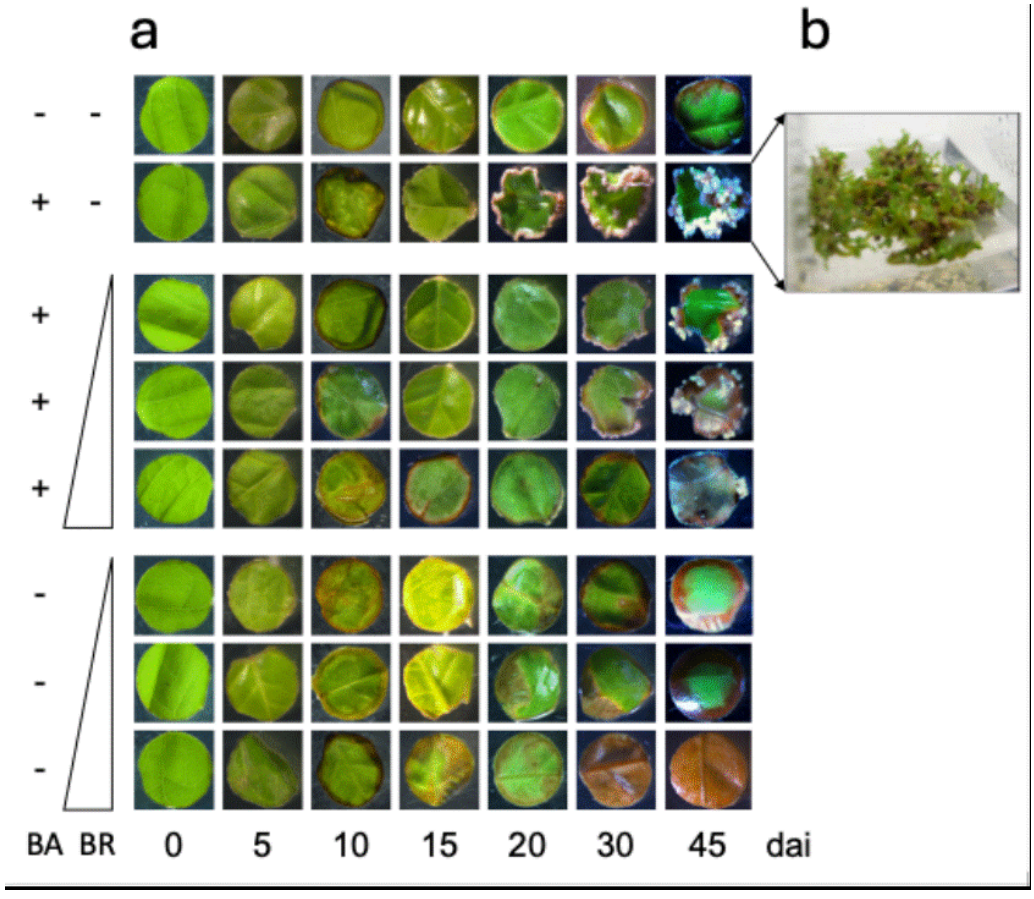

Figure 1. Effect of 22(S),23(S)-homobrassinolide on the somatic embryogenesis of *Coffea canephora*. a. Increasing concentrations of BR (triangles) were added, in the presence or absence (negative control) of 5 µM BA. Sample discs were collected after different periods. b. Seedlings develop from cotyledonal embryos after being transferred to germination media. All samples were photographed under a stereomicroscope. Images are representative of three independent experiments.

cytokinin inductor, the addition of BR was deleterious to the SE process at all concentrations used (Fig. 1, rows labeled BA-BR+).

The addition of BR to embryogenic explants abolished transcription of the Somatic Embryogenesis Receptor-Like Kinase 1 gene homolog (*ccSERK1*), as evaluated using qRT-PCR (as described by Pérez-Pascual *et al*. 2018). Expression of *SERK1* marks embryogenic competent cells in all species studied and is required to initiate the embryogenic differentiation (Schmidt *et al*. 1997, Pérez-Pascual *et al*. 2018). In the absence of BR, *ccSERK1* transcripts were detected in leaf explants from all collection periods, with peaks of expression at zero, ten, and 15 dai (Fig. 2a, panel BA+BR-, lanes zero, ten, and 15), a result that coincides with previous reports (Pérez-Pascual *et al*. 2018). When these leaf, explants were exposed to the smaller concentration of BR (0.005 µM), *ccSERK1* transcripts were barely detected in the leaf explants from the initial stages of the process, and barely increased at later embryogenesis stages (Fig. 2a, panel BA+BR+).

Deleterious effects of BR were confirmed by measuring the physicochemical parameters of the culture medium. As normal embryogenesis proceeded, values of pH dropped at 20 dai, rise at 30 dai, and then they did not change significatively in the last stages of the process (Fig. 2b, red line), while conductivity increased in a two-step behavior, with two peaks at ten and 30 dai (Fig. 2c, red line). The addition of different concentrations of BR, either alone (Fig. 2b, pink, grey, and dark blue lines) or in the presence of 5 µM BA (Fig. 2b, green, light blue, and yellow lines) produced a constant drop of pH, below those values of the normal treatment, and an increment of the conductivity values above those found in normal embryogenesis, either with (Fig. 2c, green, light blue and yellow lines) or without BA (Fig. 2c, pink, grey and dark blue lines).

The presence of stress and the external addition of different PGRs have been essential to inducing SE (Nic-Can *et al*. 2016, WójciK *et al*. 2020). The fundamental role of auxins in the induction and development of both zygotic and somatic embryogenesis differentiation has been demonstrated (Möller et al. 2017, Winnicki 2020, Wójcik *et al*. 2020, Wójcikowska *et al*. 2020, Verma *et al*., 2021). In *C. canephora*, the addition of benzyladenine to preconditioned leaf explants triggers the embryogenesis process *in vitro* and induces early expression of *ccSERK1*. When overexpressed at the initial stages of SE, *ccSERK1* induced transcription of genes encoding auxin transporters and biosynthetic enzymes, as well as early embryogenesis genes, but repressed late embryogenesis genes (Pérez-Pascual *et al*. 2018). Concerning BR, the pioneering work of Azpeitia *et al*. (2003) demonstrated that exposure of plumule explants from *C. nucifera* to the 22(S),23(S)-homobrassinolide increased their capacity to form an initial callus, embryogenic callus, and somatic embryos. In *Pinus caribaea*, Malabadi *et al*. (2011) reported the successful stimulation of embryogenesis when embryogenic

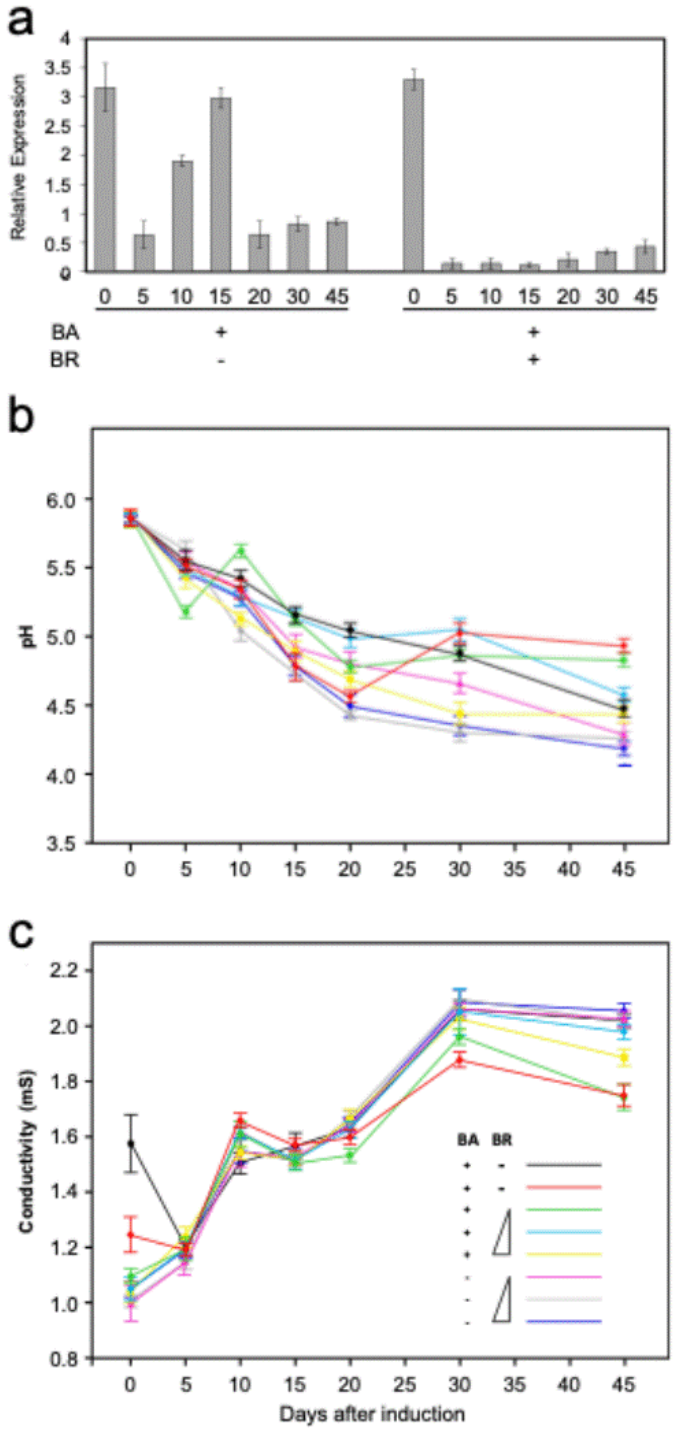

Figure 2. Effect of 22(S),23(S)-homobrassinolide on the C. canephora SERK1 homolog gene expression and biochemical parameters. a. gene expression analysis of the SERK1 gen homolog was evaluated in each treatment using real-time RT-PCR. pH (b) and conductivity (c) values were measured with a pH Meter. Each treatment is indicated with line colors in the inner square of panel c. Bars over the columns and icons represent the standard deviation of three independent experiments.

tissues derived from mature zygotic embryos were exposed to a mixture of 24-epiBrassinolide and 2,4-D. Aydin *et al*. (2006) showed that the addition of BR to *Gossypium hirsutum* calli in a medium containing BA and kinetin as embryogenic inducers increased the formation of somatic embryos and improved their transition to maturation phases, suggesting that BR and cytokinins could have a synergistic action on the regulation of gene expression that controls embryogenesis induction and transition through the maturational stages of embryogenesis. Interestingly, Chone *et al*. (2018) found that the addition of 24-epiBR in association with 2-iP to *C. arabica* explants produced 6.8 times more somatic embryos that were better morpho-histologically structured than those obtained with cytokinin alone.

However, negative effects of BR on the SE process have been observed in other species. For example, the external application of BR reduced the embryogenic competence induced by 2,4-D in *Arabidopsis thaliana* seedlings (Kwaaitaal 2007). Evidence of the present work demonstrated that brassinosteroids do not increase, but rather reduce, the embryogenic capacity of competent leaf explants of *C. canephora*, contrary to their effects observed in *C. arabica* (Chone *et al*. 2018). In *C. canephora*, gene expression of *ccSERK1* is abolished in the presence of BR; yet *SERK1* homologs can mediate brassinosteroid-dependent signaling pathways in other species (Albrecht *et al*. 2008), more experimental evidence is needed to decipher the possible interactions of BR and *ccSERK1* at early stages of *C. canephora* somatic embryogenesis. Even though *C. canephora* (a diploid species) and *C. arabica* (tetraploid) are species that belong to the same Genus, with SE processes induced both by cytokinins, they have different embryogenic capacities and biochemical requirements; in fact, the addition of an aliquot from the embryogenesis medium of *C. arabica* repressed the progression of the embryogenesis process in *C. canephora* (Nic-Can *et al*. 2015). However, there is no experimental evidence to support a role for the ploidy difference of *Coffea* species in their somatic embryogenesis behavior.

The antagonist effects of BR on the induction of somatic embryogenesis, found in this work and different species demonstrate that the mechanisms by which BR regulates embryogenic competence *in vitro* are not completely conserved, even within species of the same Genus.

AUTHOR'S CONTRIBUTION

RSP: Methodology, writing–original draft preparation, writing–review and editing. DJG: Methodology, writing–original draft preparation, writing–review and editing. VMJ: Conceptualization, writing–original draft preparation, writing–review and editing. RMH: writing–original draft preparation, writing–review and editing. JJZA: Conceptualization, formal analysis, investigation, methodology, visualization, writing–original draft preparation, writing–review and editing.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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