SCIENTIA PLENA
www.scientiaplena.org.br
VOL. 20, NUM. 10 2024
doi: 10.14808/sci.plena.2024.104901

Direct somatic embryogenesis on *Coffea arabica* L. explants previously subjected to water restriction

Embriogênese somática direta em explantes foliares de *Coffea arabica* L. previamente submetidos à restrição hídrica

J. A. S. Almeida[1]*; W. C. F. Neofiti[1]; P. E. R. Marchiori[2]; G. S. Giomo[1]

[1]*Centro de Café "Alcides Carvalho", Instituto Agronômico de Campinas, 13020-902, Campinas - São Paulo, Brasil*
[2]*Departamento de Biologia, Setor de Fisiologia Vegetal, Universidade Federal de Lavras, 37200-000, Lavras - Minas Gerais, Brasil*

**julieta.almeida@sp.gov.br*
(Recebido em 05 de maio de 2024; aceito em 25 de setembro de 2024)

Somatic embryogenesis can occur either directly or indirectly. However, *Coffea arabica* genotypes respond inefficiently to direct somatic embryogenesis. In this way, explants of this species form small embryogenic structures (pre-embryogenic masses) and a few somatic embryos. Studies have shown that the intensity of the osmotic potential can either favor or hinder the somatic embryogenesis response in different species. Plant tissue subjected to water restriction presents a reduction in osmotic potential. Therefore, this study aimed to evaluate the direct somatic embryogenesis response of leaf explants from four genotypes of *C. arabica,* which were previously subjected to 0, 1, 2, 3, 4 and 8 days of water restriction. At time 0, the explants had an average humidity of 70 %, which decreased to approximately 40 % after 8 days of water restriction. Somatic embryo formation occurred in explants subjected to up to 3 days of water restriction and there was no response in those with 4 and 8 days without hydration. Explants with low intensity osmotic potential of up to -0.8 MPa formed somatic embryos, whereas this response was reduced or inhibited in those with a more negative osmotic potential. However, explants with an osmotic potential equal to or less than -1.6 MPa formed larger embryogenic structures, which is desirable as they also give rise to somatic embryos. Thus, the direct somatic embryogenesis response of *C. arabica* is influenced by the intensity of the osmotic potential in the explant tissue.
Keywords: relative water content, somatic embryo, osmotic potential.

A embriogênese somática pode ocorrer de forma direta e indireta. No entanto, genótipos de *Coffea arabica* respondem ineficientemente à embriogenese somática direta. Nesta via, explantes desta espécie formam estruturas embriogênicas (massa pré-embriogênica) de tamanho pequeno e poucos embriões somáticos. Estudos mostram que a intensidade do potencial osmótico pode favorecer ou prejudicar a resposta de embriogênese somática em diferentes espécies. Tecido vegetal submetido a restrição hídrica apresenta redução do potencial osmótico. Assim, este estudo teve como objetivo avaliar a resposta de embriogênese somática direta de explantes foliares de quatro genótipos de *C. arabica* previamente submetidos a 0, 1, 2, 3, 4 e 8 dias de restrição hídrica. No tempo 0, os explantes apresentaram em média de 70 % de umidade e após 8 dias de restrição hídrica esta estava em torno de 40 %. A formação de embriões somáticos ocorreu em explantes submetidos a até 3 dias de restrição hídrica e não houve resposta naqueles com 4 e 8 dias sem hidratação. Explantes com potencial osmótico de baixa intensidade, até -0,8 Mpa formaram embriões somáticos e esta resposta foi reduzida ou inibida naqueles mais negativos. Porém, explantes com potencial osmótico igual ou menor que -1,6 MPa formaram estruturas embriogênicas de maior tamanho, o que é desejável já que estas também formam embriões somáticos. Assim, a resposta da via direta de *C. arabica* é influenciada pela intensidade do potencial osmótico do tecido do explante.
Palavras-chave: conteúdo relativo de água, embrião somático, potencial osmótico.

1. INTRODUCTION

Brazil is a leading global producer, exporter, and consumer of *Coffea arabica*, with a harvest of 58.082 thousand bags of processed coffee [1]. Hybrids of this species also occupy a significant place in the coffee productive chain, aiming to explore the genetic variability resulting from crosses, to produce, for example, special coffees with optimum beverage quality. However, these hybrids must be multiplied vegetatively to preserve their genetic pattern since

the plants resulting from the germination of their seeds lose their special characteristics due to genetic segregation. Vegetative multiplication of *C. arabica* hybrids can be done by micro-propagation, via somatic embryogenesis.

Somatic embryogenesis forms somatic embryos without the fusion of gametes and can occur from different cell types, depending on the species [2, 3]. This process can occur indirectly or directly with or without the formation of calluses, respectively. *C. arabica* can form somatic embryos by indirect [4, 5], direct [6] or both pathways. However, the occurrence of the direct pathway is impaired in this genotypes of this species since they form a reduced number of somatic embryos in a long time period or the response is inhibited [7]. On the other hand, stress factors can stimulate or hinder the occurrence of the somatic embryogenesis process [8-11].

During the process of starting *in vitro* cultivation installation, the plant tissues suffer stress due to surface disinfection and cuts to obtain explants. The time required to carry out the initial steps may vary depending on the logistics of tissue collection, the morphological characteristics of the species and the degree of difficulty in disinfecting it. Thus, during the initial stages, the tissue loses moisture as from collection up to the introduction of the explants into the culture medium [12]. This loss of moisture can cause cellular damage that subsequently affects their somatic embryogenesis response [13].

A large number of results were found in the literature from the application of water stress induced by osmotic agents added to the culture medium, which, depending on the type and concentration, promoted or inhibited the somatic embryogenesis response in different species [14-17].

Furthermore, the reports showed that somatic embryogenesis responses may vary according to the physiological conditions of the explant donor mother plant [18]. If the mother plant is under water stress, its explants will tend to have a lower osmotic potential, which may influence their somatic embryogenesis response. All these observations lead us to the conclusion that tissues previously subjected to lack of water may promote the somatic embryogenesis response as verified in other studies, showing that this process is positively induced by water stress [11]. So, depending on the intensity of the stress, it can cause cell death or metabolic reorganization that leads to cellular readaptation, which may or may not induce the formation of somatic embryos [8-10, 19]. Thus, it is possible that *C. arabica* explants subjected to water stress will present changes in their osmotic potential component, that may eventually promote or inhibit their direct somatic embryogenesis response. It was also noted that there was a lack of reports on the somatic embryogenesis response in explants previously subjected to water restriction, before their introduction into the culture medium.

Considering these observations, the objective of this study was to evaluate the direct somatic embryogenesis response of *C. arabica* leaf explants previously subjected to water restriction.

2. MATERIAL AND METHODS

Leaves collected up to the third pair of plagiotropic branches belonging to the middle region of the tree canopy were used in this study, taken from the following adult *C. arabica* hybrid plants producing fruits with optimal beverage quality: H8089 (Catuaí Vermelho x Geisha), H8105 (Catuaí Vermelho x BA10), H8427 (Acaiá x BA10) and from the cultivar Mundo Novo IAC 376-4. All plants were maintained in the field in an experimental area of the Santa Elisa Farm of the Instituto Agronômico de Campinas, located in the municipality of Campinas, state of São Paulo, Brazil, which is located at a latitude of -22.8750816º, longitude of 47.0753271º and elevation of 696 meters, during the years 2011-2012.

The leaves were collected in the morning and immediately disinfected. For this, the leaves were first washed with a detergent solution and rinsed under running water. They were then immersed in a 2 % sodium hypochlorite solution for twenty-five minutes and rinsed in distilled water three times. The disinfected leaves were then placed in a moist chamber with about 80 % humidity for twenty-four hours and again immersed in a 2 % sodium hypochlorite solution. After disinfecting the leaves, rectangular explants (1.5 x 2.0 cm) were obtained in a laminar flow chamber.

Previously disinfected foliar explants from the four genotypes were submitted to complete moisture restriction for 0, 1, 2, 3, 4 and 8 days. The explants were placed in 12 cm diameter Petri dishes, lined with two sheets of filter paper, both previously autoclaved, without the addition of

any moisture, and wrapped in plastic film. During the application of the treatments, the plates with the explants were maintained in a plant growth incubator at 25 ºC in the absence of light. At the end of the application of each hydric restriction period, the explants were evaluated for their relative water content (RWC) and osmotic potential.

The RWC was determined according to Jamaux et al. (1997) [20], with modifications. 1 cm diameter leaf disks were obtained from the foliar explants of the four genotypes soon after being submitted to the treatments of 0, 1, 2, 3, 4 and 8 days of water restriction. For this analysis the leaf disks were first weighed to obtain the fresh mass (fm), then placed in 20 mL flasks with 2 mL of distilled water to reach maximum turgor, and then left for 24 hours under low light conditions at room temperature. The disks were then weighed to obtain the turgid mass (tm), followed by drying in an incubator at 60 ºC to determine the dry mass (dm). The data obtained were used to calculate the RWC as follows: RWC = 100 (fm-dm)/(tm-dm).

The leaf explant samples were then exposed to the moisture restriction treatments, leaf discs (6 mm in diameter) were excised and frozen in liquid nitrogen for 30 seconds followed by thawing at room temperature. The samples were then placed inside C-52 chambers (Wescor Inc., Logan, UT) attached to a Dew Point Micro-voltmeter HR-33T (Wescor Inc., Logan, UT, USA). The osmotic potential measurements were obtained using the dew point method [21].

For the induction of direct somatic embryogenesis, a cultivation medium with ½ the normal salts concentration of the MS [22] medium was used, with the addition of 20 gL^{-1} sucrose and 30 µM 6-BA (6-benzyladenine) [23]. A cultivation medium with ½ the salts concentration of the MS medium and the addition of 20 gL^{-1} sucrose but with no plant growth regulator was used for the germination of the somatic embryos. This medium was also used for the growth and development of the embryogenic axes. The pH values of all the cultivation media were adjusted to pH 5.8 and then jellified with 2 gL^{-1} Phytagel and autoclaved at 121 ºC and 1.5 atm of pressure for twenty minutes. The explants remained in the direct pathway induction medium for up to 210 days, during which time they formed somatic embryos. Immediately after this period, the embryos were transferred to the germination medium, remaining there for 60 days, when they reached the embryonic axis stage. The embryonic axes were then transferred to the plant growth and development medium, where they remained for approximately 120 days until they reached the plant stage.

An experiment was carried out using the explants of the four genotypes submitted to water restriction treatments, which were then cultivated in direct somatic embryogenesis induction medium. In a second experiment, explants of the four genotypes were cultivated in direct pathway induction medium with the addition of 0 and 2 % Polyethylene glycol 6000 (PEG 6000). The osmotic potential was determined using the Osmometer equipment. For this purpose, samples of the culture medium were used, with and without the addition of PEG 6000. The explants were inoculated with the adaxial face in contact with the surface of the cultivation medium (30 mL) contained in 150 mL transparent glass flasks and maintained in the dark at 25 ºC for 210 days to form the somatic embryos.

Somatic embryos developed from the explants were inoculated into the germination culture medium. Subsequently, the embryogenic axes were transferred to the plant growth medium for the plant growth and development phase. For both phases, the bottles with embryos and or embryogenic axes were kept at 25º in the presence of light for 16 hours, illuminated by cool, white, fluorescent lamps with light intensity of 4.000 lux, followed by 8 hours of continuous darkness. When the *in vitro* plants had more than four pairs of leaves and the presence of roots they were transferred to the acclimatization phase.

The treatments applying direct somatic embryogenesis were evaluated in terms of the number of somatic embryos formed and an estimation of the size of the embryogenic structures. Each treatment consisted of 13 repetitions each with 2 foliar explants, adopting a completely random experimental design. To determine the RWC, for each water restriction time, three leaf disks were analyzed.

For the first experiment, a regression analysis was used to verify the relationship between the number of somatic embryos formed and the water restriction treatments. The results for the RWC, osmotic potential and the number of somatic embryos formed in the second experiment were submitted to analyses of variance and the Tukey test ($p<0.05$) to compare the means, using the GENES statistic software [24].

3. RESULTS AND DISCUTION

Leaf explants of four *C. arabica* genotypes were previously subjected to 0, 1, 2, 3, 4 and 8 days of water restriction and, after applying the treatments, immediately transferred to the direct somatic embryogenesis induction medium. The RWC evaluation showed that, in general, all the genotypes presented a reduction in moisture content with increase in the number of days the explants were submitted to moisture restriction (Figure 1). On day 0, most explants had an RWC above 70 % and on the eighth day they had moisture contents around 50 % for all treatments.

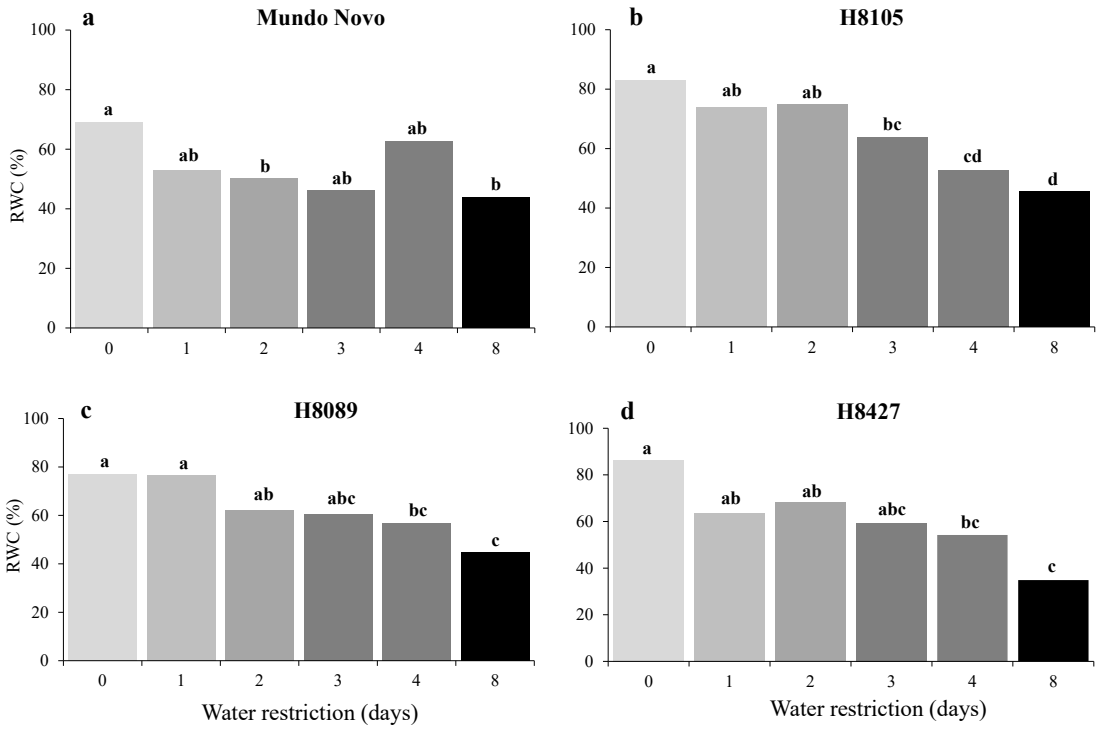

Figure 1. Determination of the relative water content (RWC) of the Coffea arabica genotype explants submitted to different water restriction times and maintained at 25ºC under light-absence conditions. Coefficient of variation= Mundo Novo: 12.29; H8105: 12.156; H8089: 18.7357; H8427: 18.57. For all treatments n= 3.

The explants of the four genotypes were also evaluated for their osmotic potential, which generally showed an increasing negative intensity as the water restriction exposure time increased (Figure 2). Explants at time 0, with no water restriction treatment, presented osmotic potentials of -0.8, -0.6, -1.2 and -0.8 MPa, respectively for the cultivars Mundo Novo (Figure 2a), H8105 (Figure 2b), H8089 (Figure 2c) and H8427 (Figure 2d). These results show that the osmotic potential value varied between the genotypes, even though the plants had the same size and were developed in the same location. Liu et al. (2003) [25] evaluated the leaf osmotic potential of 104 plant species, also finding differences, with 75 % of these showing responses between -1.01 and -3.0 MPa, with extremes ranging from -6.54 MPa in *Caragama microphylla* at -0.44 MPa in *Digitaria ischaemeum*.

The osmotic potential values of the explants studied showed a small reduction after 1 day of lack of water, with most genotypes showing responses between -1.3 and -1.7 MPa (Figure 2). From the 4th day onwards the reduction in osmotic potential was more significant and on the eighth day it was more negative for the genotypes H8427 (Figure 2d) and the cultivar Mundo Novo (Figure 2a) and less negative for H8089 (Figure 2c) and H8105 (Figure 2b), with readings of, respectively, -3.1; -3.0; -2.0 and -2.3 MPa.

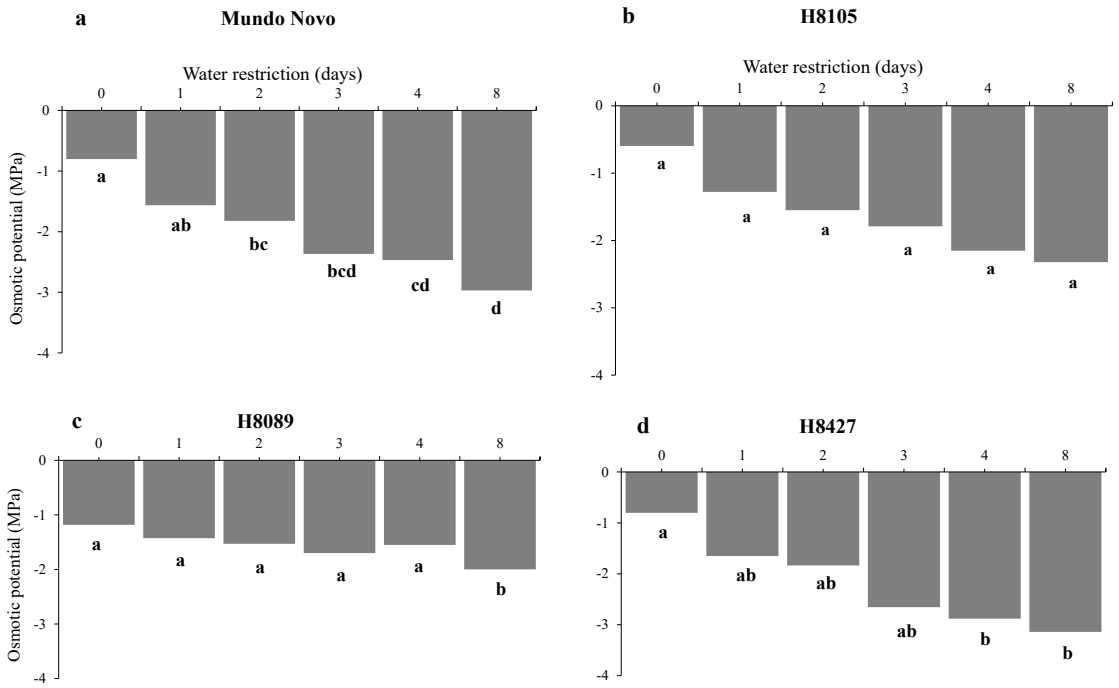

Figure 2. Determination of the osmotic potential (MPa) of the Coffea arabica genotype explants submitted to different water restriction times and maintained at 25ºC under light-absence conditions. Coefficient of variation= Mundo Novo: 19.86; H8105: 58.89; H8089: 14.770; H8427: 34.42. For all treatments n= 3.

Considering the responses of each genotype treated, the Mundo Novo cultivar was shown to present a more negative osmotic potential on the 8th day of water restriction, and this treatment did not differ statistically on days 3 and 4 (Figure 2a). The genotype H8105 showed no statistical differences between the water restriction treatments (Figure 2b). For H8089, only the treatment with 8 days of water restriction showed a different value from the other times tested (Figure 2c). For H8427, the treatments with 4 and 8 days of water restriction responded with significantly more negative osmotic potential values than the control but did not differ from those of the times of 1, 2, 3 and 4 days with lack of water (Figure 2d). The RWC and osmotic potential results indicated that the *C. arabica* explants had a significant loss of moisture as from the 4th day of lack of water, but this reduction occurred slowly, because up to the 3rd day the tissues had approximately 60 % of RWC.

Immediately after applying the water restriction treatments, explants from all genotypes were transferred to the direct somatic embryogenesis induction medium (Figure 3). The formation of somatic embryos only occurred in explants subjected to up to 3 days of water restriction, showing no response in those with 4 and 8 days of water restriction. However, the number of somatic embryos formed was greater for explants with no water restriction for most genotypes, followed by those with 1 day of water restriction, while the responses were smaller or absent in the other treatments (Figure 3).

The genotypes showed different responses, the cultivar Mundo Novo forming a greater number of somatic embryos than the hybrids. In the control treatment, the cultivar Mundo Novo formed 302 somatic embryos (Figure 3a) followed by the hybrids H8105, H8089 and H8427, which formed 27, 8 and 5 somatic embryos, respectively (Figures 3b, 3c and 3d). After one day of hydric restriction, the explants of all the genotypes showed a reduction in response, the cultivar Mundo Novo and H8089, both producing 63 somatic embryos and the hybrids H8105 and H8427, respectively 24 and 0. On the second day of treatment the production reduced significantly again, the cultivar Mundo Novo still producing more embryos followed by H8089, H8105 and H8427, respectively, with 24, 14, 6 and 0 somatic embryos. On the third day production was further reduced, the genotype H8089 forming 8 embryos and 1 to H8105, whereas the cultivar Mundo Novo and H8427 failed to respond, and on the fourth and eighth days of treatment, none of the genotypes responded.

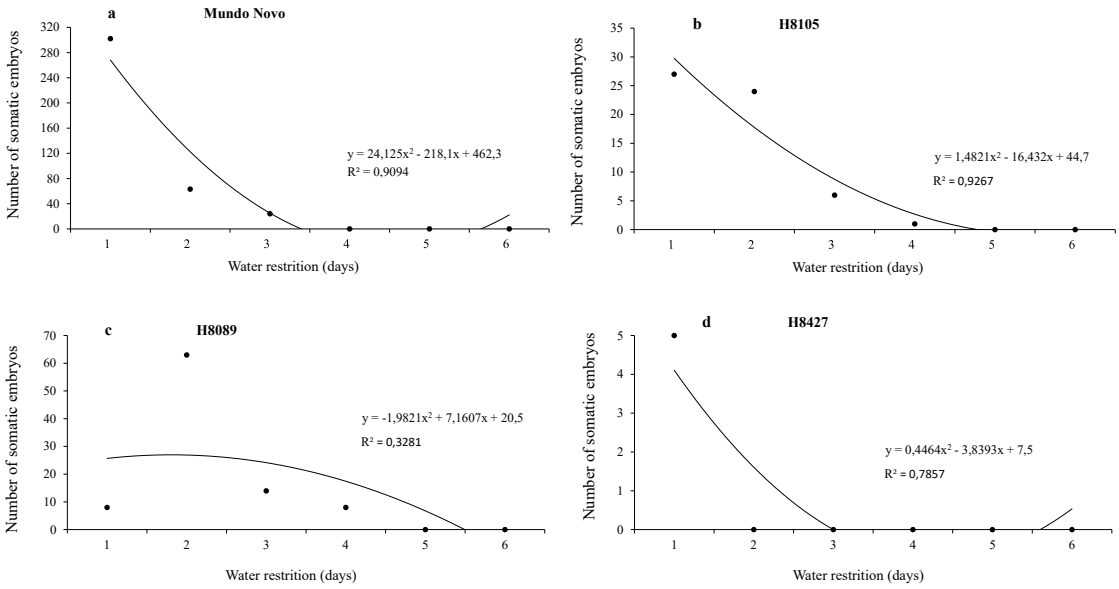

Figure 3. Number of somatic embryos formed via direct somatic embryogenesis on leaf explants of four Coffea arabica genotypes previously submitted to different water restriction times and maintained at 25 ºC in the absence of light, 210 after the beginning of cultivation.

The edges of *C. arabica* leaf explants subjected to the direct route form somatic embryos plus small structures called embryogenic structures, pre-embryogenic mass, that are also capable of forming somatic embryos on their surface [6, 26]. This study showed that, in general, explants subjected to water restriction formed larger embryogenic structures than those from the control treatment (Figure 4). The Mundo Novo cultivar formed structures measuring 5 mm in the 3 day water restriction treatment, followed by 2.5 mm for the 0 and 1 day treatments, and less than 2 mm for the other treatments (Figure 4a). On the other hand, the genotype H8105 formed structures measuring up to 2.5 mm in the 0, 1 and, 2-day water restriction treatments and the other treatments up to 1 mm (Figure 4b). Explants of genotypes H8089 and H8427 subjected to all times of water restriction formed larger embryogenic structures than those from the control treatment (Figures 4c, 4d).

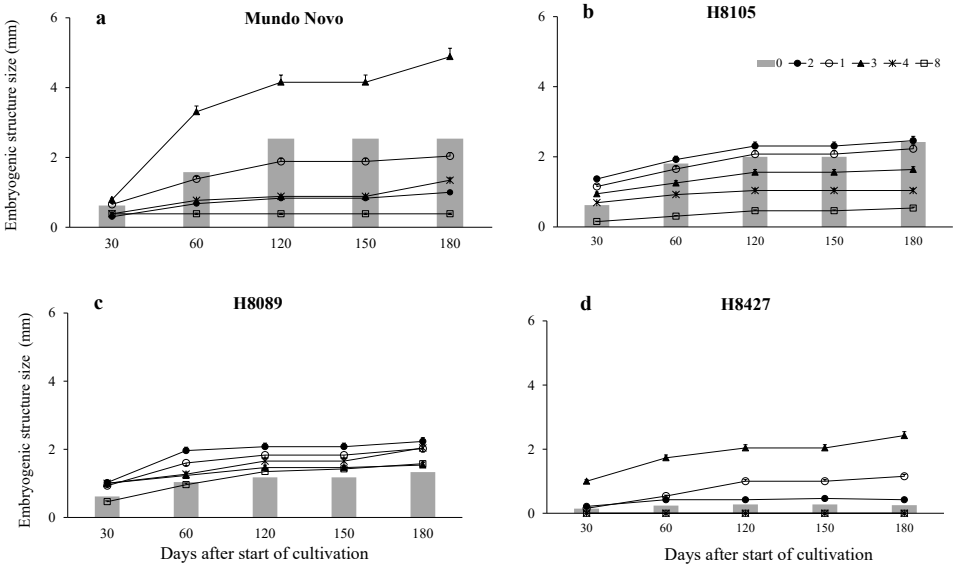

Figure 4. Embryogenic structures formed via direct somatic embryogenesis on leaf explants of four Coffea arabica genotypes previously submitted to different water restriction times, maintained at 25ºC in the absence of light, 210 hours after the beginning of cultivation. The size data of the embryogenic structures from each treatment were analyzed in relation to the mean standard deviation.

In the second experiment, explants of the four genotypes were subjected to direct somatic embryogenesis in the presence of osmotic potentials of -0.374 and -0.412 MPa induced, respectively, by the addition of 0 and 2 % PEG 6000 to the culture medium (Figure 5). The explants formed a greater number of somatic embryos in the presence of the more negative osmotic potential, -0.412 MPa. The genotypes H8089 (Figure 5c) and H8427 (Figure 5d) formed the greatest number of somatic embryos, followed by the cultivar Mundo Novo (Figure 5a) and H8105 (Figure 5b).

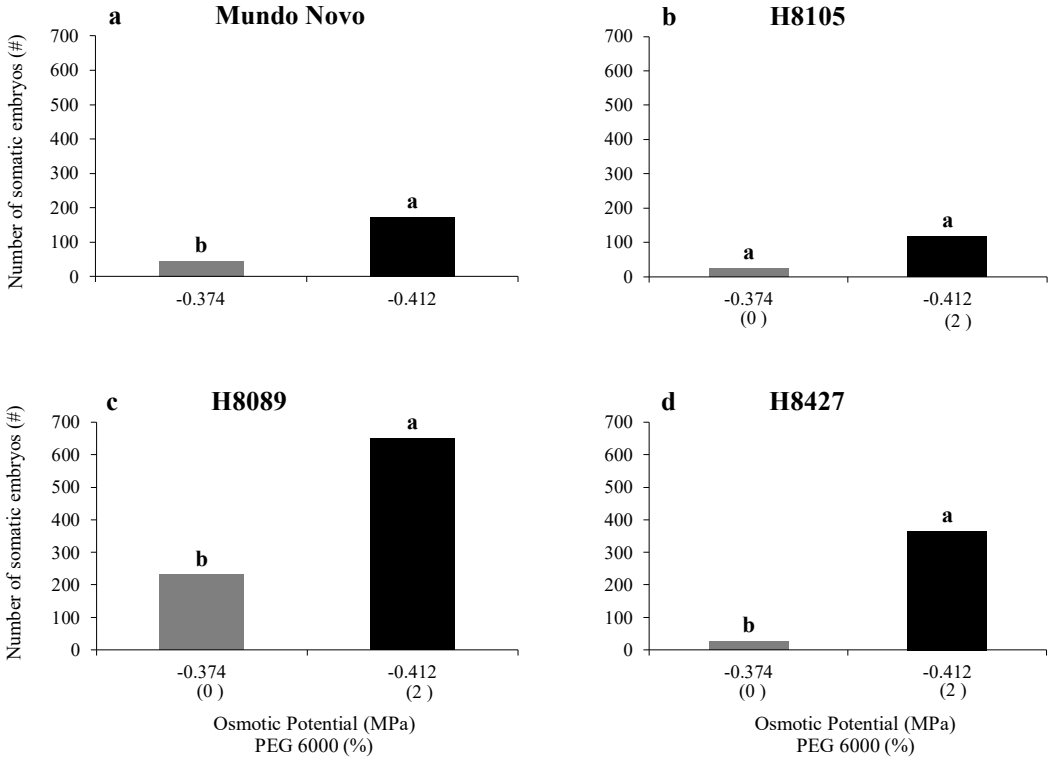

Figure 5. Number of somatic embryos formed on leaf explants of four Coffea arabica genotypes in direct somatic embryogenesis induction culture medium with the addition of 0 and 2% PEG 6000, maintained at 25 ºC in the absence of light, 210 hours after the start of the experiment. $CV_{Mundo\ Novo}$ *= 127.50, n=13;* CV_{8105} *= 185.04, n=13;* CV_{8089} *= 37.95, n=13;* CV_{8427} *= 68.97, n=13.*

The somatic embryos originating from the explants (Figure 6a) of all experiments completed their development, germinated, and reached the *in vitro* plant stage (Figure 6b).

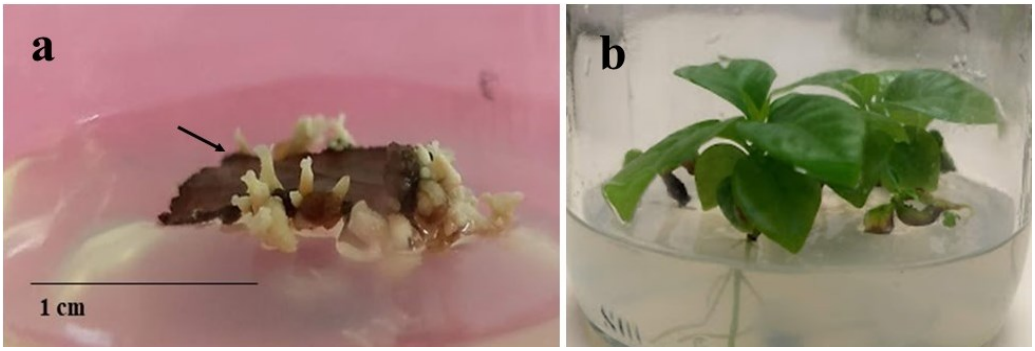

Figure 6. Direct somatic embryogenesis of leaf explants from the Coffea arabica Mundo Novo cultivar. ***a****. The arrow points to somatic embryos formed at the edges of the explant by the direct route in the absence of light at 25 ˚C.* ***b****. In vitro plants developed from somatic embryos.*

The results of this study showed that the formation of somatic embryos in the four *C. arabica* genotypes via the direct route was greater in control explants with higher moisture content, and reduced or inhibited in those that were more dehydrated. This indicates that direct somatic embryogenesis in *C. arabica* is favored in explants with a higher moisture content. Similarly, Yildiz and Özgen (2004) [27] found that flax hypocotyl explants showed higher shoot regeneration rates when immersed in distilled water before cultivation, suggesting that *in vitro* responses are favored in hydrated tissues. These observations highlight the importance of controlling the moisture content of explants before their introduction into *in vitro* culture, since the number of somatic embryos decreased with increased exposure to water restriction (Figure 3). Tissues with longer extraction times from the mother plant can generate explants with less responsiveness to somatic embryogenesis due to moisture loss. Thus, it is recommended to minimize the time interval between collecting the explant tissue and introducing it into the culture medium. The literature shows that explants tend to lose moisture during the disinfection and acquisition phases [12, 13].

The results of this study also showed that the direct somatic embryogenesis response of the four *C. arabica* genotypes was related to the intensity of the osmotic potential of the explant tissue before its introduction *in vitro*. Initially, it was found that the process was completely inhibited by more negative values of between -1.6 MPa and -3.1 MPa. Thus, an osmotic potential of intensity equal to or more negative than -1.6 MPa represented a situation of osmotic stress for the explants of this species. This response is in line with studies in the literature that show that osmotic stress can induce or inhibit the somatic embryogenesis response in different species or lead to cell death [8, 9, 11, 19]. Although the explants in this study were subjected to different periods of water restriction before being inserted into the culture medium, it was noted that these treatments were effective in affecting their subsequent direct somatic embryogenesis response (Figure 3). According to Pasternak and Steinmacher (2024) [3], stress factors should be applied for a short time and removed shortly afterwards, since their continuous application harms the subsequent development of the treated tissue.

Interestingly, in this study, explants with an osmotic potential more negative than -1.6 MPa did not form somatic embryos but were able to develop embryogenic structures, pre-embryogenic mass, on their edges. Almeida et al. (2022) [17] also found that *C. arabica* leaf explants formed embryogenic structures in the presence of an osmotic potential of up to -2.635 MPa induced by the addition of PEG 6000 to the culture medium. However, these observations suggest that in the presence of osmotic stress the explant cells stop forming embryos and develop embryogenic structures. These observations suggest that osmotic stress may affect the hormonal metabolism of explant cells, especially in relation to auxins, which are associated with cellular reprogramming [11].

Another aspect verified from the results of this study was that the greatest formation of somatic embryos occurred in explants from the control treatment which were those with a lower osmotic potential of up to -0.8 MPa. In the literature, most results regarding the effect of osmotic potential on somatic embryogenesis correspond to studies with the addition of an osmotic agent to the culture medium [14, 28, 29]. A scarcity of studies on the assessment of the osmotic potential of explant tissue before its introduction into the culture medium was also noted. In addition, the literature shows there is no fixed osmotic potential intensity that influences tissue responses *in vitro*, and that this varies between different species and tissue types. Almeida et al. (2022) [17] found that *C. arabica* explants formed somatic embryos by the direct route in media with up to -0.691 MPa induced by the addition of PEG 6000 to the culture medium. An osmotic potential of -2.0 MPa in the cultivation medium favored the direct organogenesis of the aerial part of *Elaeis guineensis* [30], whilst apical tissue fragments of *Metroxylon sagu* did not develop at -0.3 MPa but showed optimum growth at -0.5 MPa [31]. Calluses of two soybean cultivars showed a progressive reduction in the growth rate at -0.6 and -0.7 MPa [32]. Valencia-Lozano et al. (2021) [16] found that the osmotic stress induced by the addition of 9 g/L gelrite favored the conversion of 95.9% of *Coffea* somatic embryos into plants, as compared to the control, which only reached 39.34%.

Yildiz et al. (2016) [33] stated that the *in vitro* regeneration response was favored when the explant tissue presented a reduction in osmotic potential, which tends to favor the absorption of components from the culture medium and plant growth regulators. Furthermore, considering the number of somatic embryos formed by the four genotypes, the capacity to respond to direct

somatic embryogenesis was shown to be different between them. H8089 was more responsive to direct somatic embryogenesis, exhibiting the formation of somatic embryos on explants submitted to hydric restriction times of 0, 1, 2 and 3 days, followed by the cultivar Mundo Novo and H8105 exhibited their formation for treatments of 0, 1 and 2 days, while the hybrid H8427 only formed somatic embryos for the control treatment. In general, it was noted that the H8089 explants maintained less negative osmotic potentials during most of the hydric restriction times (Figure 2). Thus, a low intensity osmotic potential seems to favor the capacity for direct somatic embryogenesis in *C. arabica* explants. This observation was reinforced by the results of the second experiment since explants cultured in media with an osmotic potential of -0.412 MPa showed greater somatic embryo formation than those in -0.374 MPa. Thus, the results obtained suggest the existence of a range of low intensity osmotic potentials that may favor the occurrence of the direct pathway in *C. arabica* explants.

4. CONCLUSION

Leaf explants of *C. arabica* subjected to water restriction show a reduction in RWC and have a more negative osmotic potential, depending on the water restriction exposure time. The occurrence of direct somatic embryogenesis in this species is favored in explants with higher humidity while this response is reduced or inhibited in those with lower humidity. Furthermore, in this species, the occurrence of the direct pathway is associated with low-intensity osmotic potentials of up to -0.8 MPa, whereas this process is reduced or inhibited by more negative potentials.

5. ACKNOWLEDGEMENTS

The authors are grateful to the *Fundação de Apoio À Pesquisa do Estado de São Paulo* (FAPESP) for its financial support, Process 11/10090-1.

6. BIBLIOGRAPHIC REFERENCES

1. Companhia Nacional de Abastecimento (CONAB). Acompanhamento da safra brasileira – Café: 1º Levantamento de café – Safra 2024 [Internet]; 2024 Jan 18 [cited 2024 Nov 01]. Available from: https://www.conab.gov.br/info-agro/safras/cafe/boletim-da-safra-de-cafe
2. Horstman A, Bemer M, Boutilier K. A transcriptional view on somatic embryogenesis. Regeneration. 2017 Dec;4:201-16. doi: 10.1002%2Freg2.91
3. Pasternak TP, Steinmacher D. Plant growth regulation in cell and tissue culture In Vitro. Plants. 2024 Dec;13:327. doi: 10.3390/plants13020327
4. Söndahl MR, Sharp WR. High frequency induction of somatic embryos in cultured leaf explants of *Coffea arabica* L. Z. Pflanzenphysiol. 1977 Mar;81:395-408.
5. Almeida JAS, Silvarolla MB, Fazuoli LC, Stancato GC. Embriogênese somática em genótipos de *Coffea arabica* L. Coffee Sci. 2008 Jul;3:143-51.
6. Almeida JAS, Leal RR, Carmazini VCB, Salomon MV, Guerreiro-Filho O. Characterization of the morphological events in the somatic embryogenesis in the direct somatic of *Coffea arabica* L. genotypes. Plant Cell Biotechnol Mol Biol. 2016 ;17:393-403.
7. Nic-Can GI, Galaz-Ávalos RM, De-La-Pena C, Alcazar-Magaña A, Wrobel K, Loyola-Vargas VM. Somatic embryogenesis: Identified factors that lead to embryogenic repression. A case of species of the same genus. Plos One. 2015 Jun;10:1-21. doi: 10.1371/journal.pone.0126414
8. Zavattiere MA, Frederico AM, Lima M, Sabino R, Arnholdt-Shmitt B. Induction of somatic embryogenesis as example of stress-related plant actions. Electron J Biotechnol. 2010 Jul;13:1-9. doi: 10.4067/S0717-34582010000100012
9. Fehér A. Somatic embryogenesis - Stress-induced remodeling of plant cell fate. Biochim Biophys Acta. 2015 Jul;1849(4):385-405. doi: 10.1016/j.bbagrm.2014.07.005
10. Pais MS. Somatic embryogenesis induction in woody species: The future after omics Data Assessment. Front Plant Sci. 2019 Mar;10:240. doi: 10.3389/fpls.2019.00240
11. Ibáñez S, Carneros E, Testilano PS, Perez-Perez JM. Advances in plant regeneration: Shake, rattle and roll. Plants. 2020 Jul;9(7):897. doi: 10.3390/plants9070897
12. Elhiti M, Stasolla C. Transduction of signals during somatic embryogenesis. Plants. 2022 Jan;11(2):178. doi: 10.3390/plants11020178.

13. Nic-Can GI, Avilez-Montalvo JR, Aviles-Montalvo RN, Márquez-López RE, Mellado-Mojica E, Galaz-Ávalos RM, et al. The relationship between stress and somatic embryogenesis. In: Loyola-Vargas VM, Ochoa-Alejo N. Somatic embryogenesis: fundamental aspects and applications. Switzerland: Springer International Publishing; 2016. p. 151-70. doi: 10.3390/plants10071467
14. Eliane MT, Modeste KK, Andre SB, Edmond KK, Mongomake K. Effect of water stress induced by polyethylene Glycol 6000 on somatic embryogenesis in Cocoa (*Theobroma cacao* L.). Agric Sci. 2019 Sep;10:1240-54. doi: 10.4236/as.2019.109092
15. Martínez-Santos E, Cruz-Cruz CA, Spinoso-Castillo JL, Bello-Bello JJ. In vitro response of vanilla (*Vanilla planifolia* Jacks. ex Andrews) to PEG-induced osmotic stress. Sci Rep. 2021 Nov;11:22611. doi: 10.1038/s41598-021-02207-0
16. Valencia-Lozano E, Ibarra JE, Herrera-Ubaldo H, Folter S, Cabrera-Ponce JL. Osmotic stress-induced somatic embryo maturation of coffee *Coffea arabica* L., shoot and root apical meristems development and robustness. Sci Rep. 2021 May;11:9661. doi: 10.1038/s41598-021-88834-z
17. Almeida JAS, Neofiti WCF, Giomo GS. Osmotic potential intensity in the control of direct somatic embryogenesis in coffee tree. Pesqu Agropecu Bras. 2022 Jul;57:e02947. doi: 10.1590/S1678-3921.pab2022.v57.02947
18. Molina DM, Aponte ME, Cortina H, Moreno G. The effect of genotype and explant age on somatic embryogenesis of coffee. Plant Cell Tissue Organ Cult. 2002 Nov;71:117-23. doi: 10.1023/A:1019965621041
19. Spinoso-Castillo JL, Bello-Bello JJ. In vitro stress-mediated somatic embryogenesis in plants. In: Ramírez-Mosqueda MA. Somatic embryogenesis. Methods and protocols. New York (US): Humana Press; 2022. p. 223-35. doi: 10.1007/978-1-0716-2485-2_16
20. Jamaux I, Steinmetz A, Belhassen E. Looking for molecular and physiological markers of osmotic adjustment in sunflower. New Phytologist. 1997 Jun;137:117-27. doi: 10.1046/j.1469-8137.1997.00817.x
21. Pearson CH, Kirkham MB. Osmotic potential of wheat grown with fertilizer placed in strips or broadcasted. Fertilizer Res. 1980 Dec;1:227-234. doi: 10.1007/BF01074195
22. Murashige T, Skoog FA. Revised medium for rapid growth and bioassays with tabacco tissue cultures. Physiol Plant.1962 Jul;15:473-97. doi: 10.1111/j.1399-3054.1962.tb08052.x
23. Almeida JAS, Silvarolla MB. Induction of somatic embryos of *Coffea arabica* genotypes by 6-benzyladenine. Int J Plant Dev Biol. 2009 Apr;1:5-9.
24. Cruz CD. Programa Genes: estatística experimental e matrizes. Viçosa (MG): UFV; 2006.
25. Liu MZ, Jiang GM, Li YG, Niu SL, Gao LM, Ding L, et al. Leaf osmotic potentials of 104 plant species in relation to habitats and plant functional types in Hunshandak Sandland, Inner Mongolia, China. Trees. 2003 Jan;17:554-60. doi: 10.1007/s00468-003-0277-8
26. Ferrari IF, Marques GA, Sachetti Junior WL, Biazotti BB, Passos MP, de Almeida JAS, et al. Comparative ontogenesis of *Coffea arabica* L. somatic embryos reveals the efficiency of regeneration modulated by the explant source and the embryogenesis pathway. In Vitro Cell Dev Biol Plant. 2021 Jun;57:796-810. doi: 10.1007/s11627-021-10200-5
27. Yildiz M, Özgen M. The Effect of a submersion pretreatment on in vitro explant growth and shoot regeneration from hypocotyls of flax (*Linum Usitatissimum*). Plant Cell Tiss Org Cult. 2004 Apr;77:111-5. doi: 10.1023/B:TICU.0000016493.03592.c3
28. Kacem NS, Delporte F, Muhovski Y, Djekoun A, Watillon B. In vitro screening of durum wheat against water-stress mediated through polyethylene glycol. J Genet Eng & Biotechnol. 2017 Jun;15:239-47. doi: 10.1016/j.jgeb.2017.04.004
29. Chagas K, Cipriano JLD, Lopes JC, Schmildt ER, Otoni WC, Alexandre RS. The effects of an osmoregulator, carbohydrates and polyol on maturation and germination of 'Golden THB' papaya somatic embryos. An Acad Bras Ciênc. 2018 Oct;90(4):3433-47. doi: 10.1590/0001-3765201820171035
30. Romyanon K, Watana K, Pichakum A, Mosaleeyanon K, Kirdmanee C. Adjustment of medium composition and iso-osmotic potential in direct-shoot organogenesis produces true-to-type oil palm (*Elaeis guineenses* Jacq.) plantlets. Hortic Environ Biote. 2017 Dec;58:601-12. doi: 10.1007/s13580-017-0254-6
31. Novero A, Delima AG, Acaso J, Baltores LM. The influence of osmotic concentration of media on the growth of Sago Palm (*Metroxylon sagu* Rottb.) in vitro. Aust J Crop Sci. 2010 Aug;4(6):453-6.
32. Sakthivelu G, Devi MKD, Giridhar P, Rajasekaran T, Ravishankar GA, Nedev T, et al. Drought-induced alterations in growth, osmotic potential and in vitro regeneration of soybean cultivars. Gen Appl Plant Physiol. 2008 Jan;34(1-2):103-12.
33. Yildiz M, Darcin ES, Beyaz R. Water stress hinders in vitro regeneration of plants. In: Rahman IMM, Begum ZA, Hasegawa H, editors. Water stress in plants. London: IntechOpen [Internet]; 2016. doi: 10.5772/64664