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ARTICLE

In vitro seed germination and seedling development of Rio Grande cherry (*Eugenia involucrata* DC.)

Germinação *in vitro* de sementes e desenvolvimento de plântulas de cerejeira-do-Rio-Grande (*Eugenia involucrata* DC.)

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Abstract: Trees play an important role in urban landscapes by offering ecological and functional benefits such as heat mitigation and biodiversity support. Native fruit trees, such as the Rio Grande cherry (*Eugenia involucrata* DC.), may have great potential to be used in this context, by providing food for local fauna and promoting ecological balance, and easily adapt to this environment. On the other hand, propagation is limited by its annual fruiting cycle, recalcitrant seeds, and the low efficiency of clonal propagation methods. Tissue culture offers a promising alternative for large-scale seedling production using *in vitro* germinated seedlings for micropropagation. This study aimed to optimize *in vitro* establishment of Rio Grande cherry by evaluating seed preparation and cultivation conditions to enhance *in vitro* germination and nursery tree production. Factors such as light conditions, tegument removal, MS salt concentrations, sucrose concentrations, and seed fractioning were evaluated in several distinct experiments. High germination and seedling development rates were achieved by removing the tegument and cultivating seeds in the dark. Using a complete MS medium and supplementation of 15 g L^{-1} of sucrose also enhanced seedling development. Despite fractioned seeds having some germination potential, seedling development was negatively affected.
Keywords: Atlantic Forest, Myrtaceae, native species, tissue culture, woody plant.

Resumo: Árvores são essenciais nas paisagens urbanas, oferecendo benefícios ecológicos e funcionais, como mitigação do calor e suporte à biodiversidade. Frutíferas nativas, como a cerejeira-do-Rio-Grande (*Eugenia involucrata* DC.), podem apresentar elevado potencial para utilização neste contexto, ao fornecer alimento para a fauna local e promover o equilíbrio ecológico, e facilmente adaptar-se a esses ambientes. Por outro lado, sua propagação é limitada pelo ciclo anual de frutificação, sementes recalcitrantes e baixa eficiência de métodos de propagação clonal. O cultivo *in vitro* surge como uma alternativa promissora para a produção em larga escala de mudas, utilizando-se de plântulas germinadas *in vitro* para micropropagação. Este estudo buscou otimizar o estabelecimento *in vitro* de cerejeira-do-Rio-Grande, avaliando o preparo das sementes e as condições de cultivo para potencializar a germinação *in vitro* e a produção de mudas. Fatores como condições de luz, remoção do tegumento, concentrações de sais MS, concentrações de sacarose e fracionamento das sementes foram avaliados em diversos experimentos distintos. Altas taxas de germinação e desenvolvimento de plântulas foram alcançadas removendo o tegumento e cultivando sementes no escuro. A utilização do meio MS completo e a suplementação de 15 g L^{-1} de sacarose também favoreceram o desenvolvimento das plântulas. Embora sementes fracionadas tenham apresentado algum potencial germinativo, o desenvolvimento das plântulas foi afetado negativamente.
Palavras-chave: cultura de tecidos, espécie nativa, Mata Atlântica, Myrtaceae, planta lenhosa.

Introduction

Trees are essential natural elements in urban landscapes, offering both visual and functional benefits (Wolf et al., 2020). Urban tree planting provides numerous advantages, including food production, heat mitigation, and aesthetic enhancement. Fruit trees hold a particularly important role by supplying fruit and pollen to insects and birds, thereby supporting the health of urban ecosystems (Liang and Huang, 2023). Additionally, they contribute to the achievement of at least nine Sustainable Development Goals (SDGs) established by the United Nations (UN) (Brito and Borelli, 2020).

When planning urban tree planting, native species should be prioritized for their superior contribution to ecological balance through fruit production that supports local fauna. Their natural adaptation to regional edaphoclimatic conditions also promotes healthier and more robust growth, ensuring greater success in adapting to urban environments (Braga et al., 2024).

The Rio Grande cherry (*Eugenia involucrata* DC.), a native Myrtaceae species, holds significant potential for urban arborization. This tall, deciduous tree (8 – 15 m) is native to southeastern and southern Brazil, naturally occurring from Minas Gerais to Rio Grande do Sul. Commonly found in the Atlantic Forest, domestic orchards, and rural properties, it produces white, hermaphroditic flowers in spring, and its sweet, wine-red fruits benefit both local fauna and human consumption (Lorenzi, 2014; Santos, 2022).

Despite its great potential, propagation of this species is still primarily through seeds (Lorenzi, 2014), which is limited by its biological characteristics, as it fruits only once a year, from October to November (Danner et al., 2010). Additionally, Rio Grande cherry seeds are recalcitrant (Maluf et al., 2003), which reduces their storage viability, and conventional clonal propagation methods have proven unsatisfactory.

To support large-scale seedling production for this species, tissue culture stands out as a viable approach, utilizing *in vitro* germinated seedlings as explant sources for micropropagation. The use of this type of explant source has been successfully reported for various native woody species of the Myrtaceae family, including cambuci *(Campomanesia phaea* (O. Berg) Landrum] (Demétrio et al., 2021), pitanga (*Eugenia uniflora* L.) (Stefenon et al., 2020), and Rio Grande cherry (*Eugenia involucrata* DC.) (Oliveira Junior et al., 2024).

This study reports the results of a series of experiments involving the effect of light, tegument removal, salts, sucrose, and seed fractioning on seed germination and seedling development on the *in vitro* establishment of Rio Grande cherry.

Material and Methods

Plant material

Mature fruits were harvested from four-year-old plants maintained in an experimental orchard at the Areão Experimental Farm, Luiz de

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Queiroz College of Agriculture (ESALQ/USP), Piracicaba, São Paulo, Brazil (22°41'31.7" S, 47°38'41.6" W), during the 2019 season. Following collection, the fruits were taken to the laboratory, manually pulped, washed under running water, and air-dried at room temperature for 24 hours.

For disinfestation, seeds were immersed in 70% ethanol for 1 minute, followed by a 15-minute soak in sodium hypochlorite solution (1.25% active chlorine) with three drops of Tween 20®, under agitation. Seeds were then rinsed five times with autoclaved distilled water in a laminar flow chamber. In all the experiments, seeds were selected based on minimum size, excluding those less than 8 mm in length.

Effect of light condition and tegument removal on seed germination and seedling development

Treatments included seeds kept either under light (70 µmol m^{-2} s^{-1}) with a 16-hour photoperiod at 25 ºC or in the dark (BOD chamber, 24 hours at 26 ºC ± 1), with or without tegument removal. Teguments were carefully removed with the use of a scalpel. After the disinfestation procedure, seeds were inoculated into test tubes (25 x 150 mm), containing complete MS basal medium (Murashige and Skoog, 1962) supplemented with 30 g L^{-1} sucrose and 2 g L^{-1} Phytagel™ (Sigma-Aldrich). The pH of the medium was adjusted to 5.8 before autoclaving (20 min at 120 ºC, 1 atm). Each tube was sealed with a plastic lid and wrapped with polyvinyl chloride (PVC) film.

Seed germination (%) and seedling development (%) were assessed every 7 days until the 35th day. After 35 days, seedling height (cm) and the number of nodes were recorded. Seedling development (%) was calculated by the number of completely developed seedlings (with complete roots and aerial parts) divided by the number of introduced seeds. Data on germination and seedling development were used to calculate the germination speed index (GSI) and emergence speed index (ESI). GSI was determined by the formula GSI = G1/N1+G2/N2+…+Gn/Nn, where Gn is the number of germinated seeds at each assessment, and Nn is the number of days until the assessment. The ESI was calculated as ESI = E1/N1 + E2/N2 + ... + En/Nn, where En represents the number of seedlings that developed aerial part at each assessment, and Nn is the number of days until the assessment (Maguire, 1962).

The experimental design was completely randomized in a 2 x 2 factorial scheme (light condition x tegument removal), with five replications, ten test tube per replication, and one seed per test tube.

Effect of MS media salt and sucrose concentration on seed germination and seedling development

For this experiment, the seed teguments were carefully removed with the use of a scalpel, followed by seed disinfestation, and inoculation according to the treatments, which varied by MS media salt concentrations (complete MS, ½ MS, and ¼ MS) and sucrose concentrations (15 g L^{-1} and 30 g L^{-1}). The media were supplemented with 2 g L^{-1} Phytagel™, with the pH adjusted to 5.8 before autoclaving (20 min at 120 ºC, 1 atm). Seeds were inoculated in test tubes (25 x 150 mm), sealed with a plastic lid, wrapped with polyvinyl chloride (PVC), film and maintained in a BOD chamber in the dark at 26 ºC ± 1 for the duration of the experiment. Seed germination (%) and seedling development (%) were assessed every 7 days until the 35th day. Seedling development (%) was calculated by the number of completely developed seedlings (with complete roots and aerial parts) divided by the number of introduced seeds. After 35 days, seedling height (cm), number of nodes and apical necrose (%) were recorded. The germination speed index (GSI) and emergence speed index (ESI) were calculated as previously described.

The experimental design was completely randomized in a 3 x 2 factorial scheme (MS medium salts x sucrose concentration), with five replications, ten test tubes per replication, and one seed per test tube.

Effect of seed fractioning on germination and seedling development

The seed teguments were carefully removed with the use of a scalpel, followed by seed disinfestation and fractionation under a laminar flow chamber using a scalpel and tweezers, according to the treatments: whole seeds without fractioning; seeds cut in half at the hilum, following the placental scar mark (Silva et al., 2005); and seeds cut into four parts, with both transverse and longitudinal cuts. After fractioning, seeds were placed in Magenta™ containers (77 x 77 x 97 mm) containing complete MS basal medium supplemented with 30 g L^{-1} sucrose and 2 g L^{-1} Phytagel™ (Sigma-Aldrich). The pH of the medium was adjusted to 5.8 before autoclaving (20 min at 120 ºC, 1 atm). Containers were sealed with a plastic lid, wrapped with polyvinyl chloride (PVC) film, and maintained in a BOD chamber in the dark at 26 ºC ± 1.

Seed germination (%) and seedling development (%) were assessed every 7 days until the 35th day. After this period, the experimental units were transferred to a growth room under light (70 µmol m^{-2} s^{-1}) with a 16-hour photoperiod at 25 ºC, where they remained until the 60th day. Seedling development (%) was calculated by the number of completely developed seedlings (with complete roots and aerial parts) divided by the number of introduced explants (seed fractions). After 60 days, germination (%), seedling development (%), seedling height (cm), and number of nodes were recorded. The germination increment factor (GIF), and complete seedling production factor (CSPF) were also calculated as described by Silva (2003). For GIF, the average number of fractions initiating germination per seed was determined (GIF = ngf/ns), where ngf is the number of germinable fractions and ns is the number of seeds placed for germination. For CSPF, the average number of fractions producing complete seedlings was calculated (CSPF = nfp/ns), where nfp is the number of fractions producing complete seedlings and ns is the number of seeds placed for germination.

The experimental design was completely randomized, with three treatments (whole seeds without fractioning; seeds cut in half at the hilum, and seeds cut into four parts), ten replications (Magenta™ container), each containing five seeds or five seed fractions per container.

Statistical analyses

Normality and homogeneity assumptions were tested. Once these assumptions were met, two-way and one-way ANOVA were conducted, followed by Tukey's post-hoc test ($p < 0.05$). Germination percentage data were transformed using the arcsine transformation. For the germination increment factor (GIF) and complete seedling production factor (CSPF), the Kruskal-Walli's test was applied, followed by Dunn's post-hoc test ($p < 0.05$).

Results

No contamination or oxidation was observed in the seeds during the carry-out of the experiments.

Effect of light condition and tegument removal on seed germination and seedling development

In vitro seed germination of Rio Grande cherry was significantly affected by light conditions and presence or absence of seed tegument on the 7 and 14 days after *in vitro* culture (Table 1). As early as 7 days after seed inoculation, seeds without tegument began to germinate. Moreover, seeds in dark without tegument, demonstrated a higher germination percentage, compared to those exposed to light treatment (Table 1).

Table 1. Seed germination (%) after 7 and 14 days of *in vitro* cultivation (DIC) of Rio Grande cherry seeds in relation to light condition and tegument removal.

Tegument	Light condition			
	7 DIC		14 DIC	
	Light	Dark	Light	Dark
With	$0.0^{Aa} \pm 0.0$	$0.0^{Ab} \pm 0.0$	$0.0^{Ab} \pm 0.0$	$2.0^{Ab} \pm 2.0$
Without	$22.0^{Ba} \pm 5.8$	$60.0^{Aa} \pm 3.2$	$70.0^{Ba} \pm 6.3$	$96.0^{Aa} \pm 2.4$
Mean	20.5		41.5	

Mean values ± standard error. Values followed by the same uppercase letters, within rows, and by the same lowercase letters, within columns, are not significantly different by Tukey's test ($p < 0.05$).

Along the evaluation period, there was no differences observed in the interaction of tegument removal and light conditions on seed germination. However, the isolated effect was significant. The tegument removal increased seed germination percentage for the from the 21st day to the end of the experiment (35th day) (Table 2). The same pattern was observed for seeds maintained in the dark (Table 3).

Table 2. Seed germination (%) after 21, 28, and 35 days of *in vitro* cultivation (DIC) of Rio Grande cherry seeds in relation to tegument removal.

Tegument	21 DIC	28 DIC	35 DIC
With	$2.0^{b} \pm 1.3$	$19.0^{b} \pm 4.3$	$39.0^{b} \pm 5.3$
Without	$95.0^{a} \pm 2.7$	$95.0^{a} \pm 2.7$	$95.0^{a} \pm 2.7$
Mean	48.5	57.0	67.0

Mean values±standard error. Values followed by the same letter, within columns, are not significantly different by Tukey's test ($p < 0.05$).

Table 3. Seed germination (%) after 21, 28, and 35 days of *in vitro* cultivation (DIC) of Rio Grande cherry seeds in relation to light condition.

Light condition	21 DIC	28 DIC	35 DIC
Light	$45.0^{b} \pm 15.0$	$51.0^{b} \pm 13.3$	$60.0^{b} \pm 10.6$
Dark	$52.0^{a} \pm 16.0$	$63.0^{a} \pm 12.7$	$74.0^{a} \pm 9.2$
Mean	48.5	57.0	67.0

Mean values ± standard error. Values followed by the same letter, within columns, are not significantly different by Tukey's test ($p < 0.05$).

Seedling development began 14 days after seed inoculation. Seeds kept in dark without tegument began to develop aerial parts, differing from the other conditions (Table 4), and sustained the best results through the 35th day of evaluation. Seeds with tegument, regardless of light conditions, showed a low percentage of seedling development (Table 4).

In general, the presence of seed tegument arrested or inhibited seed germination and development, and the absence of light induced seedling etiolation (Fig. 1)

Table 4. Seedling development (%) after 14, 21, 28, and 35 days of *in vitro* cultivation (DIC) of Rio Grande cherry seeds in relation to light condition and tegument removal.

Tegument	Light condition							
	14 DIC		21 DIC		28 DIC		35 DIC	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
With	$0.0^{Aa} \pm 0.0$	$0.0^{Ab} \pm 0.0$	$0.0^{Ab} \pm 0.0$	$0.0^{Ab} \pm 0.0$	$0.0^{Ab} \pm 0.0$	$2.0^{Ab} \pm 2.0$	$2.0^{Ab} \pm 2.0$	$2.0^{Ab} \pm 2.0$
Without	$0.0^{Ba} \pm 0.0$	$18.0^{Aa} \pm 3.7$	$20.0^{Ba} \pm 11.4$	$74.0^{Aa} \pm 6.8$	$58.0^{Ba} \pm 9.7$	$86.0^{Aa} \pm 4.0$	$68.0^{Ba} \pm 8.0$	$90.0^{Aa} \pm 3.2$
Mean	4.5		23.5		36.0		40.5	

Mean values ± standard error. Values followed by the same uppercase letters, within rows, and by the same lowercase letters, within columns, are not significantly different by Tukey's test ($p < 0.05$).

Fig. 1. *In vitro* germination and seedling development of Rio Grande cherry (*Eugenia involucrata* DC.) seeds in different light conditions (light and dark) and tegument removal (with or without) at 35 days after cultivation. A – Light with tegument. B – Dark with tegument. C – Light without tegument. D – Dark without tegument. Bar = 1 cm.

Seeds kept in dark without tegument resulted in higher values of seedling height and number of nodes. High germination and seedling development percentages observed in seeds kept in dark without tegument resulted in superior germination speed index (GSI) and emergence speed index (ESI) values (Table 5).

Table 5. Seedling height (SH), number of nodes (NN), germination speed index (GSI), and emergence speed index (ESI) after 35 days of *in vitro* cultivation of Rio Grande cherry seeds in relation to light condition and tegument removal.

Tegument	Light condition							
	SH (cm)		NN		GSI		ESI	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
With	$0.1^{Ab} \pm 0.1$	$0.4^{Ab} \pm 0.4$	$0.2^{Ab} \pm 0.2$	$0.8^{Ab} \pm 0.8$	$0.09^{Ab} \pm 0.01$	$0.17^{Ab} \pm 0.03$	$0.01^{Ab} \pm 0.01$	$0.01^{Ab} \pm 0.01$
Without	$2.1^{Ba} \pm 0.2$	$5.8^{Aa} \pm 0.1$	$3.7^{Ba} \pm 0.2$	$6.2^{Aa} \pm 0.2$	$0.75^{Ba} \pm 0.07$	$1.13^{Aa} \pm 0.02$	$0.26^{Ba} \pm 0.04$	$0.45^{Aa} \pm 0.02$
Mean	2.1		2.7		0.53		0.18	

Mean values ± standard error. Values followed by the same uppercase letters, within rows, and by the same lowercase letters, within columns, are not significantly different by Tukey's test ($p < 0.05$).

Effect of MS media salt and sucrose concentration on seed germination and seedling development

The MS media salt and sucrose concentrations did not affect the germination and seedling development of Rio Grande cherry. However, considering only the isolated effect of MS salt concentrations, a difference in seedling development was recorded between the 14th and 28th day of evaluation. During this period, best seedling development was achieved in seeds cultivated in ½ MS and ¼ MS salt concentrations (Table 6). At the end of the experiment (35th day), no differences were observed in seedling development according to different MS salt concentrations (Table 6).

Table 6. Seedling development (%) after 14, 21, 28, and 35 days of *in vitro* cultivation (DIC) of Rio Grande cherry seeds in relation to MS media salt concentration.

Culture media	14 DIC	21 DIC	28 DIC	35 DIC
MS	$49.0^{b} \pm 5.6$	$87.0^{b} \pm 2.1$	$92.0^{b} \pm 2.0$	$96.0^{a} \pm 1.6$
½ MS	$61.0^{ab} \pm 5.0$	$94.0^{ab} \pm 2.2$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$
¼ MS	$70.0^{a} \pm 6.0$	$95.0^{a} \pm 2.2$	$98.0^{a} \pm 1.3$	$99.0^{a} \pm 1.0$
Mean	60.0	92.0	96.6	98.3

Mean values ± standard error. Values followed by the same letter, within columns, are not significantly different by Tukey's test ($p < 0.05$).

The higher seedling development percentages observed with ½ MS and ¼ MS resulted in a better emergence speed index (ESI) compared to the use of complete MS (Table 7). On the other hand, the complete MS salt medium significantly induced the highest number of nodes, and ¼ MS reduced seedling height. Additionally, the ¼ MS salt medium negatively affected the seedlings by increasing the apical necrosis percentage (Table 7).

Considering the isolated effect of sucrose in the culture media, the concentration of 15 g L^{-1} resulted in higher values of seedling height and germination speed index (GSI) (Table 8).

Effect of seed fractioning on germination and seedling development

The germination potential and seedling development of *in vitro* Rio Grande cherry seeds were compared between whole and fractionated seeds (Fig. 2).

Table 7. Seedling height (SH), number of nodes (NN), emergence speed index (ESI), apical necrosis (AP) percentage after 35 days of *in vitro* cultivation of Rio Grande cherry seeds in relation to MS media salt concentration.

Culture media	SH (cm)	NN	ESI	AP (%)
MS	$4.9^{a} \pm 0.1$	$6.1^{a} \pm 0.2$	$0.55^{b} \pm 0.01$	$23.0^{a} \pm 4.0$
½ MS	$4.7^{a} \pm 0.2$	$5.3^{b} \pm 0.2$	$0.62^{a} \pm 0.01$	$29.0^{a} \pm 5.0$
¼ MS	$4.2^{b} \pm 0.1$	$4.7^{b} \pm 0.2$	$0.63^{a} \pm 0.01$	$57.0^{b} \pm 5.0$
Mean	4.6	5.4	0.60	36

Mean values±standard error. Values followed by the same letter, within columns, are not significantly different by Tukey's test ($p < 0.05$).

Table 8. Seedling height (SH) and emergence speed index (ESI) after 35 days of *in vitro* cultivation of Rio Grande cherry seeds in relation to sucrose concentration.

Sucrose (g L^{-1})	SH (cm)	ESI
15	$4.9^{a} \pm 0.1$	$0.62^{a} \pm 0.01$
30	$4.3^{b} \pm 0.1$	$0.59^{b} \pm 0.01$
Mean	4.6	0.60

Mean values±standard error. Values followed by the same letter, within columns, are not significantly different by Tukey's test ($p < 0.05$).

Fig. 2. *In vitro* germination and seedling development of Rio Grande cherry (*Eugenia involucrata* DC.) seeds according to the fractioning (whole seeds; cut in half and cut into four parts) at 60 days after cultivation. A – Whole seeds. B – Seeds cut in half ai the hilum. C – Seeds cut into four parts. Bars = 1 cm.

A higher germination percentage was recorded in whole seeds, as compared to fractionated seeds, as early as 7 days after cultivation, and was significantly different until the end of the evaluation period (60^{th} day) (Table 9).

Similarly, maximum seedling development was achieved in derived seedlings from whole seeds at the end of experiment. However, during experiment evaluation, seedling development was not different between whole seeds and seeds cut in half, from the 14^{th} to the 35^{th} day of data collection (Table 10).

Table 9. Seed germination (%) after 7, 14, 21, 28, 35, and 60 days of *in vitro* cultivation (DIC) of Rio Grande cherry seeds in relation to seed fractioning.

Seed fractioning	7 DIC	14 DIC	21 DIC	28 DIC	35 DIC	60 DIC
Whole seed	$62.0^{a} \pm 7.0$	$80.0^{a} \pm 6.7$	$84.0^{a} \pm 5.0$	$84.0^{a} \pm 5.0$	$84.0^{a} \pm 5.0$	$92.0^{a} \pm 3.3$
Cut in half	$32.0^{b} \pm 6.8$	$58.0^{b} \pm 4.7$	$60.0^{b} \pm 4.2$	$60.0^{b} \pm 4.2$	$60.0^{b} \pm 4.2$	$70.0^{b} \pm 5.4$
Cut into four parts	$14.0^{c} \pm 6.7$	$28.0^{c} \pm 6.8$	$28.0^{c} \pm 6.8$	$28.0^{c} \pm 6.8$	$28.0^{c} \pm 6.8$	$28.0^{c} \pm 6.8$
Mean	36.0	55.3	57.3	57.3	57.3	63.3

Mean values±standard error. Values followed by the same uppercase letters, within rows, and by the same lowercase letters, within columns, are not significantly different by Tukey's test ($p < 0.05$).

Table 10. Seedling development (%) after 14, 21, 28, 35, and 60 days of *in vitro* cultivation (DIC) of Rio Grande cherry seeds in relation to seed fractioning.

Seed fractioning	14 DIC	21 DIC	28 DIC	35 DIC	60 DIC
Whole seed	$42.0^{a} \pm 9.6$	$50.0^{a} \pm 10.0$	$58.0^{a} \pm 9.6$	$66.0^{a} \pm 7.9$	$86.0^{a} \pm 4.3$
Cut in half	$18.0^{ab} \pm 7.0$	$28.0^{a} \pm 6.1$	$32.0^{ab} \pm 6.8$	$38.0^{a} \pm 6.3$	$52.0^{b} \pm 7.4$
Cut into four parts	$6.0^{b} \pm 4.3$	$6.0^{b} \pm 4.3$	$10.0^{b} \pm 4.5$	$14.0^{b} \pm 7.9$	$26.0^{b} \pm 7.3$
Mean	22.0	28.0	33.3	39.3	54.6

Mean values ± standard error. Values followed by the same uppercase letters, within rows, and by the same lowercase letters, within columns, are not significantly different by Tukey's test ($p < 0.05$).

Although seeds cut in half had a lower germination percentage compared to whole seeds, they achieved a germination increment factor of 1.4, representing a 48% increase in germination compared to whole seeds. However, the complete seedling production factor did not differ between treatments (Table 11). Finally, whole seeds produced taller seedlings with a greater number of nodes (Table 11).

Table 11. Germination increment factor (GIF), complete seedling production factor (CSPF), seedling height (SH), and number of nodes (NN) after 60 days of *in vitro* cultivation of Rio Grande cherry seeds in relation to seed fractioning.

Seed fractioning	GIF[1]	CSPF[1]	SH (cm)[2]	NN[2]
Whole Seed	$0.92^{b} \pm 0.03$	$0.88^{a} \pm 0.03$	$3.3^{a} \pm 0.1$	$4.0^{a} \pm 0.1$
Cut in half	$1.40^{a} \pm 0.11$	$1.08^{a} \pm 0.15$	$2.4^{b} \pm 0.4$	$2.5^{b} \pm 0.4$
Cut into four parts	$1.12^{ab} \pm 0.27$	$1.12^{a} \pm 0.27$	$1.4^{c} \pm 0.3$	$1.4^{b} \pm 0.4$
Mean	1.15	1.02	2.4	2.6

Mean values ± standard error. [1]Values followed by the same letter, within columns, are not significantly different by Dunn's test ($p < 0.05$). [2]Values followed by the same letter, within columns, are not significantly different by Tukey's test ($p < 0.05$).

Discussion

It is well established that the structure of the tegument or seed coat can inhibit germination, as it interferes with water uptake, gas exchange, and may contain chemical inhibitors, thereby creating a physical and chemical barrier to the germination process (Bewley and Black, 1982). This phenomenon is commonly observed in Brazilian native tree species (Colado et al., 2020), including members of the *Eugenia* genus such as *E. pyriformis* (Tafarel et al., 2021).

In our study, the removal of the tegument from Rio Grande cherry seeds resulted in significantly higher germination percentages by the 7th day after inoculation, along with notable seedling development observed on the 14th and 21st days of cultivation, resulting also in better GSI and ESI. In contrast, seeds with tegument exhibited low germination and seedling development, indicating that the tegument acts as a barrier to seed germination, likely due to its impermeability. These findings align with studies on *in vitro* germination of other species, such as *Passiflora edulis*, which have demonstrated that tegument removal can substantially enhance germination rates and accelerate seedling development under *in vitro* conditions (Faria et al., 2023).

In the present study, seeds without tegument and exposed to light exhibited delayed germination and seedling development. Although information on woody species is limited, this result may be attributed to the conversion of amyloplasts into chloroplasts under direct light exposure, as observed in potato (*Solanum tuberosum* L.) (Zhang et al., 2020). The presence of chlorophyll can negatively affect germination and vigor in important agronomic crop, leading to seed quality losses (França-Silva et al., 2022). In cabeludinha [*Myrciaria glazioviana* (Kiaersk.) G.M. Barroso *ex* Sobral], a native tree from the Myrtaceae family, light has been shown to negatively affect germination by causing oxidation and altering seed substances (Guimarães et al., 2018). In addition, seeds germinated without tegument in the dark had greater seedling height and number of nodes, likely due to etiolation, also referred to as ectomorphogenesis, in which the absence of light promotes elongated growth (Armarego-Marriott et al., 2020). In tissue culture, etiolation is commonly utilized as it produces larger seedlings with a greater number of nodes, which is advantageous for the micropropagation process (Maruyama et al., 2024).

The composition of culture media, including salt and sucrose concentrations, can influence seed germination and seedling development (Santana et al., 2022). In contrast, our study did not find any differences in the seed germination percentage regarding media composition. Seeds of *Eugenia* species, such as *E. pyriformis*, contain large amounts of starch, about 70% of the dry matter in seeds without tegument (Justo et al., 2007).

On the other hand, the diluted concentrations of MS medium promoted greater ESI values, which may be attributed to the osmotic potential negatively affecting seed water uptake (Reis et al., 2008), as the complete MS medium has a higher osmotic potential compared to ½ and ¼ MS. Seedling height and number of nodes were higher when the complete MS medium was used, likely due to the greater availability of nutrients.

A necrosis phenomenon was observed at the end of the experiment, which may be related to the low calcium concentration in the culture medium. The highest percentage of necrosis was observed in the ¼ MS medium, potentially due to calcium deficiency in relation to the rapid growth of etiolated seedlings. While apical necrosis has not been reported in woody species, Helms (1971) documented its occurrence during the *in vitro* germination of bean (*Phaseolus vulgaris* L.), attributing it to a lack of calcium in the culture medium.

Sucrose plays a crucial role in a variety of metabolic processes in plants due to the significant energy and carbon it provides for growth and development (Yoon et al., 2021). As the primary organic carbon source in plants, sucrose is widely utilized in tissue culture, as well as for osmotic regulation (Hao et al., 2024). However, supplementing *in vitro* cultures with high concentrations of sucrose can induce osmotic stress, which adversely affects seedling development by causing plasmolysis and dehydration (Choi and Jeong, 2002; Hazubska-Prybył et al., 2016).

In our study, supplementing the culture medium with 15 g L^{-1} sucrose enhanced *in vitro* seedling height and ESI. These results may be attributed to the negative impact of high osmotic pressure associated with elevated sucrose concentrations.

Eugenia species have been extensively studied for their potential to germinate and form seedlings from fractioned seeds (Silva et al., 2003; Silva et al., 2005; Amorim et al., 2020; Delgado et al., 2022). Although this process may resemble polyembryony, the seeds are monoembryonic, and seedling formation from fractioned seeds originates from the perivascular tissue in the apical cotyledonary region (Delgado et al., 2022).

In this study, fractioned seeds demonstrated the potential to germinate and form seedlings; however, they were inferior to whole seeds in terms of germination, seedling development percentage, seedling height, and number of nodes. This difference may be attributed to the reduction in seed reserves caused by fractioning (Gomes et al., 2016).

Seeds cut in half at the hilum showed a 48% increase in germination compared to whole seeds. Despite this, seedling development was not significantly different from that of whole seeds. The development of seedlings from fractioned seeds is not guaranteed by the germination process, as fractioned seeds contain fewer reserves to support their growth (Silva et al., 2003; Delgado and Barbedo, 2020).

Conclusions

Higher values of seed germination, seedling development, seedling height, and number of nodes of Rio Grande cherry were achieved by removing the tegument and cultivating the seeds in the dark. The complete MS culture medium and the supplementation of 15 g L^{-1} of sucrose promoted optimal *in vitro* seedling development. Although fractioned seeds of Rio Grande cherry exhibit germination potential, their seedling development is negatively affected by fractioning.

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Author Contribution

MAOJ: Investigation, Data Curation, Writing – Original Draft. **BABC**: Investigation, Data Curation. **LCLS**: Investigation, Data Curation. **RRL**: Investigation. **SMSP**: Data Curation, Formal Analysis. **FAAMF**: Conceptualization, Project Administration, Writing - Review & Editing.

Conflict of Interest

The authors have no competing interests (financial or non-financial) to disclose.

Data Availability Statement

Data will be made available upon request to the authors.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that the use of AI and AI-assisted Technologies was not applied in the writing process.

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