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DORMANCY BREAK INDUCED BY GIBBERELLIC ACID AND IN VITRO GERMINATION OF SEEDS AND ZYGOTIC EMBRYOS OF *PRUNUS CAMPANULATA* MAXIM

Quebra de dormência induzida por ácido giberélico e germinação in vitro de sementes e embriões zigóticos de Prunus campanulata Maxim

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Abstract: *Prunus campanulata* is a species largely adopted for landscape composition in Brazil with limited seedling production by seed dormancy, lacking studies about in vitro propagation. This study aims to evaluate different *P. campanulata* seed pretreatments on germination, and the effect of GA_3 on dormancy break and seedlings biometry. Seeds were processed in three treatments: complete seeds, seeds without endocarp, and isolated zygotic embryos incubated in a WPM medium containing different GA_3 concentrations (0.0, 2.0, and 4.0 mg L^{-1}). After 30 days of in vitro incubation were evaluated the germination percentage, mean germination time, percentage of normal seedlings, percentage of seedlings with leaves, and survival percentage were. Seedlings greater than 3.0 cm were selected for *ex vitro* adaptation, evaluating after 32 days the biometric variables: total length, aerial part length, root length, and the number of leaves. Complete seeds do not germinate, suggesting dormancy associated with the endocarp, whereas seeds without endocarp and isolated zygotic embryos showed the same germination rate, with no effect on GA_3. The GA_3 promoted greater seedling growth at concentrations between 1.89 mg L^{-1} for the total length of the isolated zygotic embryo and 2.24 mg L^{-1} for the length of the aerial part of seeds without endocarp. In conclusion, seed processing, i.e., removing the endocarp or isolating the zygotic embryo can overcome dormancy, improving germination and seedling production of *P. campanulata in vitro*.
Keywords: GA_3. Japanese cherry tree. *In vitro* embryos. Biometry.

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Resumo: *Prunus campanulata é uma espécie amplamente utilizada para composição de paisagens no Brasil com limitada produção de mudas por dormência de sementes, carecendo de estudos sobre propagação in vitro. Este trabalho tem como objetivo avaliar diferentes pré-tratamentos de sementes de P. campanulata na germinação e o efeito do GA_3 na quebra de dormência e na biometria de plântulas. As sementes foram processadas em três tratamentos: sementes completas, sementes sem endocarpo e embriões zigóticos isolados incubados em meio WPM contendo diferentes concentrações de GA_3 (0,0; 2,0 e 4,0 mg L^{-1}). Após 30 dias de incubação in vitro foram avaliados o percentual de germinação, o tempo médio de germinação, o percentual de plântulas normais, o percentual de plântulas com folhas e o percentual de sobrevivência. Mudas maiores que 3,0 cm foram selecionadas para adaptação ex vitro, avaliando-se após 32 dias as variáveis biométricas: comprimento total, comprimento da parte aérea, comprimento da raiz e número de folhas. Sementes completas não germinam, sugerindo dormência associada ao endocarpo, enquanto sementes sem endocarpo e embriões zigóticos isolados apresentaram a mesma taxa de germinação, sem efeito sobre GA_3. O GA_3 promoveu maior crescimento de plântulas em concentrações entre 1,89 mg L^{-1} para o comprimento total do embrião zigótico isolado e 2,24 mg L^{-1} para o comprimento da parte aérea das sementes sem endocarpo. Em conclusão, o processamento de sementes, ou seja, a remoção do endocarpo ou o isolamento do embrião zigótico pode superar a dormência, melhorando a germinação e a produção de plântulas de P. campanulata in vitro.*

Palavras-chave: *GA_3. Cerejeira japonesa. Embriões in vitro. Biometria.*

1 INTRODUCTION

Prunus campanulata Maxim. is a species of Rosaceae family (synonymous *Cerasus campanulata* (Maxim.) A.N. Vassiljeva and *Prunus cerasoides* var. *campanulata* (Maxim.) Koidz.)[1] known as Japan cherry in Brazil, Taiwan cherry and bellflower cherry in other countries. The species occurs in China, Vietnam, and Japan, in forests and valleys at altitudes ranging from 100 to 1.300 m[2]. Japan cherry has great potential and value as an ornamental tree, due to its bellflower blossoms, besides a broad adaptation to different sites, since the species occurs in varied environments in its original distribution area[3-4]. In Japan, the species is a timber source being then introduced in several countries, such as the United Kingdom, United States, Australia, New Zealand[4-5], and Brazil, where is used in parks, squares, and urban afforestation in the Southern region, flowering between June and July.

Although *P. campanulata* is considered an important species in the Southern, and its dispersion occurs mainly by seeds[6], it is referred to the species the production of a reduced number of seeds for a short period[7], associated with limited germination due to physiological dormancy of the embryo[8-9]. Dormancy is a phenome characterized by a viable seed that is unable to germinate, even under adequate environmental conditions[10] being caused by endogenous factors, requiring pre-germinative treatments[11]. The main causes of tree seed dormancy are the presence of substances in the seed coat inducing embryo dormancy and limiting its growth. Moreover, dormancy can be an association of different factors, such as a physiological immature embryo with, a hard and impermeable seed coat, when the seed is unable to absorb water and oxygen for its development[12-13].

Since dormancy set limitations to plant dispersion and seedling production, dormancy break techniques are needed, and *in vitro* vegetative propagation is an alternative for *P. campanulata*[7]. Among the advantages of this technique are embryogenesis control, the rescue of interspecific embryos, even when propagation limitations are present, haploid production, dormancy break,

and production of aseptic seedlings can enable plant rescue and production. Other advantages of *in vitro* propagation are standardization and time reduction of seed germination, easy to identify and equalize limiting factors, such as nutritional and physiological embryo issues[14-15].

Considering in vitro seed cultivation, zygotic embryos are commonly incubated in culture media containing salts, carbohydrates, and growth regulators[16]. Growth regulators are included in culture media to promote embryo germination and growth, gibberellins the most used, of which gibberellic acid (GA_3) can promote seed dormancy break and germination, since, in addition to the seed coat limitations, the hormonal concentration and balance such between the gibberellic acid and abscisic acid influence dormancy break[8,17]. It is important to highlight further, that hormonal concentration is a critical factor since it can induce toxicity for seeds, inhibiting seed germination. In addition, can induce seedling anomalies by promoting enzyme activity, that degenerates cell walls[11].

Presently, *in vitro* germination studies of *P. campanulata* are focused on tissue culture and mass propagation from axillary and adventitious buds via in vitro organogenesis[6-7]. In this way, information about the influence of gibberellic acid on dormancy break and germination of seed and zygotic embryos of *P. campanulata in vitro*, as well as on seedlings' growth is still lacking. Thus, this study aims to evaluate different *P. campanulata* seed pretreatments on germination, and the effect of GA_3 on dormancy break and seedlings biometry.

2 **MATERIAL AND METHODS**

2.1 PLANT MATERIAL

For this study, 180 fully mature fruits were collected in September, from four *P. campanulata* matrices, located on the Universidade Estadual do Centro-Oeste in the municipality of Irati, where the climate is classified as Cfb. The tests were carried out at the Silviculture Laboratory of Universidade Estadual do Centro-Oeste, Campus Irati, state of Parana (Unicentro).

2.2 SEEDS PREPARATION

The fruits were pulped and cleaned in running water to extract the seeds, being seeds separated into three processing treatments, each composed of matrices mix of 60 seeds: treatment 1 - complete seeds; treatment 2 - seeds without endocarp; and treatment 3 - isolated zygotic embryos. To remove the stony and woody integument, the seeds of treatments 2 and 3 were imbibed in filtered water for 24 hours, at room temperature. To remove the integument, pliers were used, cutting the ends of the seed.

2.3 IN-VITRO INCUBATION

For *in-vitro* incubation, processed seeds were disinfected in a laminar flow chamber, immersing seed in 70% alcohol (v/v) for one minute followed by immersion in 2.5% sodium hypochlorite (v/v) added with three drops of

surfactant Tween 20® for each 100 mL of solution, for 15 minutes, under constant agitation. Then, the seeds were washed three times in sterile deionized water[18]. After disinfestation, isolation of the zygotic embryos from the seeds of Treatment 3 was carried out in a sterile condition, maintaining part of the integument. This procedure was performed with tweezers and a scalpel, with the aid of a stereoscopic microscope with a 40x magnification.

Seeds were incubated in nutritive WPM (Wood Plant Medium) medium[19]. To the WPM medium was added myo inositol (100 mg L^{-1}); sucrose (30 g L^{-1}), and different concentrations of GA_3, comprising three treatments: 0.0, 2.0, and 4.0 mg L^{-1}. According to Faria et al.[20] and Pio et al.[21], sucrose is a source of carbohydrates for the embryo and favors the formation of the root in the seedling, that is, it favors germination.

After preparation, the medium pH was adjusted to 5.7 ± 0.1, adding to the medium melted agar at a concentration of 6 $g.L^{-1}$. The different GA_3 treatments were poured into test tubes (150 x 25 mm) sealed with a cap, each with 10 ml of medium, and sterilized in an autoclave at 120 ºC (1.5 atm) for 20 minutes. The seeds and isolated zygotic embryos were deposited in the test tubes, and incubated in a growth room, with a photoperiod of 16 hours of light and 8 hours of darkness, a temperature of 25 ± 2 ºC, and irradiance of 27 µmol m^{-2} s^{-1}.

2.4 STATISTICAL DESIGN AND ANALYSIS

The experiment was in a completely randomized design, in a factorial scheme (3 seed processing treatments x 3 GA_3 concentrations) with four replications and five seeds per replication, with each seed and isolated zygotic embryo individually incubated in the test tubes. The experiment totaled 180 seeds, 15 for each treatment. After 30 days of *in vitro* incubation, germination was evaluated by the following variables: germination percentage (G) (Equation 1), mean germination time (MGT) (Equation 2)[22], percentage of normal seedlings (NS), percentage of seedlings with leaves (SL) and survival percentage (S).

$$G = \left(\frac{\sum n_{gi}}{N}\right) \times 100 \quad \text{(Equation 1)}$$

$$MGT = \left(\sum_{i=1}^{k} n_i\, t_i\right) / \left(\sum_{i=1}^{k} n_i\right) \quad \text{(Equation 2)}$$

Where: N – number of incubated seeds; – total number of germinated seeds; t – average germination time; – number of seeds that germinate in time ; – time between the beginning of the experiment and the i-th (day or hour) observation; k – last day of observation.

After the evaluation of *in vitro* germination, seedlings that emerged and reached a length greater than 3 cm were selected for *ex-vitro* adaptation. These were transplanted into glass pots containing substrate based on bio-stabilized pine bark, previously sterilized in an autoclave at 120 ºC (1.5 atm) for 40 minutes, which were sealed with two layers of plastic film in the growth room.

After 32 days of adaptation, seedlings biometric evaluation was carried out, evaluating: total length (TL, cm), aerial part length (AL, cm), root length (RL, cm), and leaves number (LN, unit). Seedlings, obtained from in vitro germination, were evaluated in an unbalanced completely randomized design, with a variable number of repetitions per treatment, in a factorial scheme (2x3), in which Factor 1 consisted of seeds without endocarp and isolated zygotic embryos and the

Factor 2 constituted by the three concentrations of GA_3. Each seedling was considered a repeat in a total of 68 seedlings.

The data from the evaluations of *in vitro* germination and biometry of *P. campanulata* seedlings were submitted to the Levene and Bartlett test (p-value) respectively. The homogeneous variances had the effects of the treatments tested using the ANOVA F test, while those that showed heterogeneity had the original values transformed into (germination and survival percentages), (percentage of normal seedlings) and (percentage of seedlings with leaves), where: arc sen is the arcosene, x is the variable of interest and ln is the Neperian logarithm, for further analysis by the same means test.

For statistical analysis, software R[23] was used with the "ExpDes.pt" package[24] and the "car" package[25]. The treatment means were compared using the Tukey mean test, considering a significance of 5%.

To represent the relationship between GA_3 concentrations, in mg L^{-1}, and biometric variables, quadratic regression equations were adjusted. The trend curves were presented for the four variables evaluated, with the best-adjusted equation, presenting the ideal maximum point, coefficient of determination (R^2), and the standard error in percentage (S_{yx}%).

3 RESULTS

Germination was recorded from the third day after seed incubation, with no germination observed for complete seeds. The isolated zygotic embryos (ZE) promoted better germination (63.33%), percentage of normal seedlings (79%), percentage of seedlings with leaves (97%), and survival (61.67%) rates, except for the mean germination time, which was lower for seeds without endocarp (WE) (6 days) (Table 1).

After germination evaluations, no statistical differences were observed for the evaluated variables (G, MGT, NS, SL, and S) among GA_3 concentrations and for the interaction of seed processing treatments (seeds without endocarp and isolated embryos) x GA_3 (Table 2).

Regarding seedlings biometry, only seedlings showing normal development were evaluated. Seedlings from seeds without endocarp had the greatest mean for total length (9.40 cm) and aerial part length (5.55 cm). Root length (4.23 cm) and the number of leaves (3.87 units) were promoted on seedlings from the isolated embryos. However, maximum values were observed for seedlings from seeds with seed coats (removed endocarp) (Table 3).

For the seed factor, there was no statistically significant difference for the evaluated biometric variables. However, for the GA_3 factor (mg L^{-1}) there was a statistically significant difference in the variables among the concentrations, with the concentration of 2 mg L^{-1} providing greater seedling development. The seed processing treatments x GA_3 interaction was also statistically significant, with greater development of seedlings from isolated zygotic embryos at a concentration of 2 mg L^{-1} GA_3, except for the root length variable (Table 4).

Table 1 – Descriptive statistics of the variables evaluated after *in vitro* incubation of isolated zygotic embryos and seeds without endocarp of *Prunus campanulata* in different GA_3 concentrations

Statistics	Isolated zygotic embryos					Seeds without endocarp				
	G	MGT	NS	SL	S	G	MGT	NS	SL	S
Minimum	20.00	4	33.33	66.67	20.00	20.00	3	0	0	20.00
Mean	63.33	8	79.17	97.22	61.67	51.67	6	67.36	91.67	51.67
Maximum	100	16	100	100	100	100	8	100	100	100.00
Standard Error	6.44	1	7.07	2.78	6.26	7.57	0	11.44	8.33	7.57
Variation Coefficient (%)	35.20	48.02	30.93	9.90	35.14	50.76	23.20	58.85	31.49	50.76

G: percentage of germination; MGT: mean germination time; NS: percentage of normal seedlings; SL: percentage of seedlings with leaves; S: survival percentage.

Table 2 – Average germination percentage (G), average germination time (MGT), normal seedlings percentage (NS), seedlings with leaves percentage (SL), and survival percentage (S) of *Prunus campanulata* seeds without endocarp (WE) and isolated zygotic embryos (ZE) in the presence of different concentration of GA_3 *in vitro*

GA_3	G		MGT		NS		SL		S	
	WE	ZE	WE	ZE	WE	ZE	WE	ZE	**WE**	**ZE**
0 mg L^{-1}	60	75	6.16	7.59	100	93.75	100	100	60	75
2 mg L^{-1}	50	65	5.77	6.04	39.58	64.58	75	91.67	50	60
4 mg L^{-1}	45	50	6.58	10.92	62.5	79.17	100	100	45	50
Mean	51.67	63.33	6.0	8.0	67.33	79.17	91.67	97.25	51.67	61.67

Table 3 – Descriptive statistics of the biometric variables analyzed during the *in vitro* incubation of *Prunus campanulata* seedlings in different concentrations of GA_3

Statistics	Isolated zygotic embryos				Seeds without endocarp			
	TL (cm)	LA (cm)	RL (cm)	LN	TL (cm)	LA (cm)	RL (cm)	LN
Minimum	2.40	0.90	0.60	1.00	0.90	0.60	2.00	0.30
Mean	9.06	4.84	4.23	3.87	9.40	5.55	4.19	3.85
Maximum	15.20	9.90	8.50	10.00	21.70	13.40	14.00	9.20
Standard Error	0.68	0.40	0.36	0.42	0.88	0.54	0.38	0.37
Variation Coefficient (%)	41.55	46.13	47.98	60.67	56.68	59.39	54.50	59.04

TL: Total length; LA: length of the aerial part; RL: root length; LN: leaves number.

Table 4 – Summary of the analysis of variance of the biometric variables analyzed during the *in vitro* incubation of *Prunus campanulata* seedlings in different concentrations of GA_3

Variation	GL	Mean squares			
		TL (cm)	LA (cm)	RL (cm)	LN (unit)
Seeds processing	1	1.90^{ns}	8.64^{ns}	2.43^{ns}	0.25^{ns}
GA_3	2	262.99**	117.87**	29.10**	2,19**
Seeds x GA_3	2	60.73*	22.56**	9.32^{ns}	0.99**
Residue	62	12.89	4.19	3.74	0,19
Total	67	-	-	-	-
Mean		5.23	4.02	9.24	4,04
Variation Coefficient (%)		38.84	38.84	48.14	47.13
Bartlett test (*p*-value)		0.79^{ns}	0.79^{ns}	0.70^{ns}	0.18^{ns}

TL: Total length; LA: length of the aerial part; RL: root length; LN: leaves number. (*) Significant F test at 5% probability of error; (**) significant at the 1% probability of error level; (ns) not significant.

All biometric variables were represented by a polynomial second-degree equation (Figure 1 a-d). The same response was not observed for the number of leaves for seedlings from isolated zygotic embryos, represented by a linear equation explaining 99% of the data dispersion (Figure 1d). The root length showed no interaction between the factors, being evaluated only concerning GA_3 concentrations. According to the regression analysis the ideal GA_3 concentrations were: for total length 1.89 mg L^{-1} for isolated zygotic embryo, and 2.22 mg L^{-1} for seeds without endocarp; for length of aerial part 1.91 mg L^{-1} for isolated zygotic embryo, and 2.24 mg L^{-1} for seeds without endocarp; for root length 1.87 mg L^{-1} for isolated zygotic embryo, and 2.16 mg L^{-1} for seeds without endocarp; for leaf number 9.55 mg L^{-1} for isolated zygotic embryo, and 2.03 mg L^{-1} for seeds without endocarp.

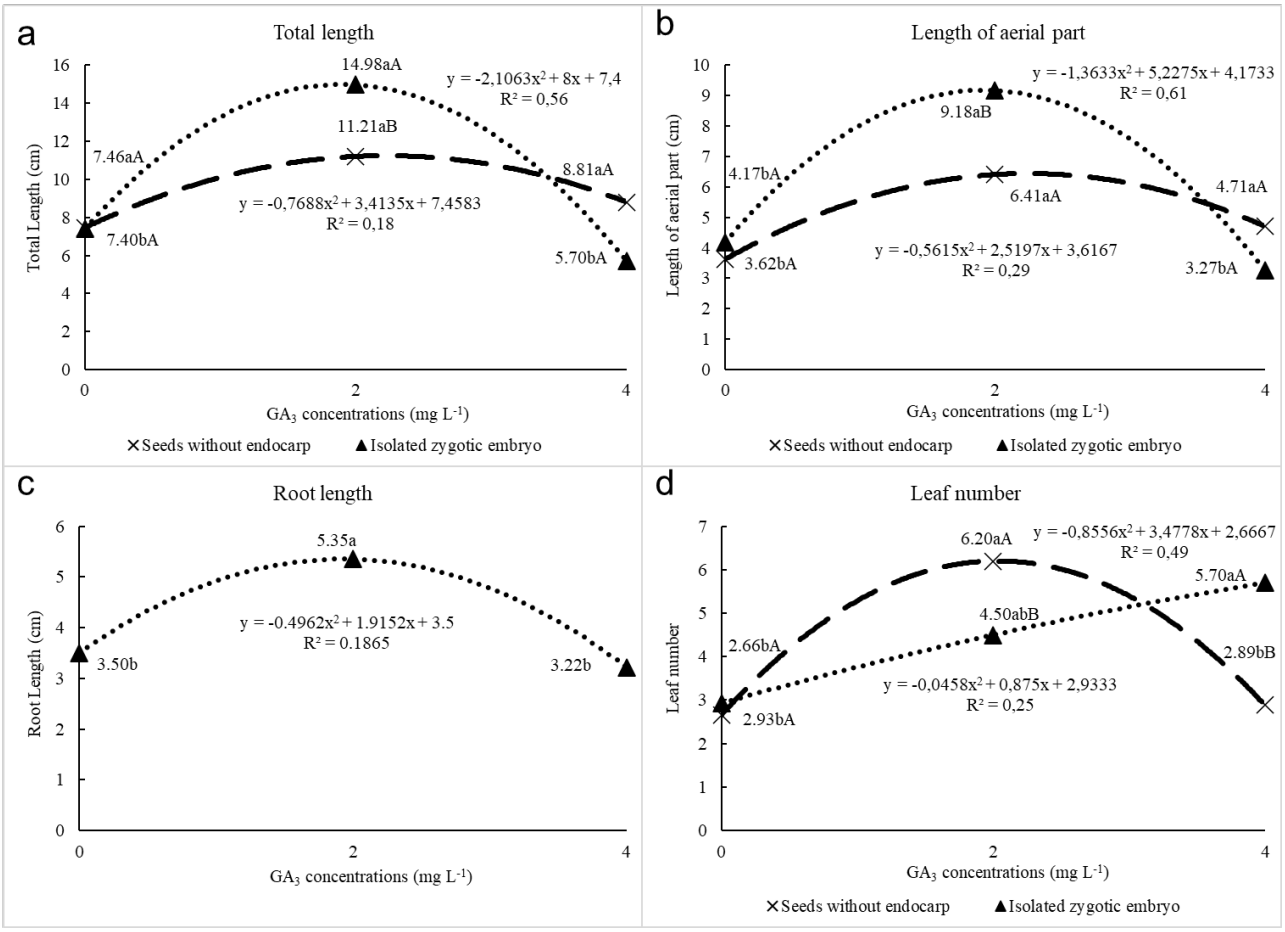

Figure 1 – Adjusted regression equations and determination coefficients (R^2) for the relationship between GA_3 concentrations (mg L^{-1}) for total length (cm) (A), length of the aerial part (cm) (B), root length (cm) (C), and leaf number (unit) (D) *in vitro* cultivation of *Prunus campanulata* seedlings. Means followed by vertical capital letters refer to the interaction of seed processing treatments within the same ga3 concentration; lowercase letters horizontally refer to the interaction of GA_3 concentrations within the same seed processing treatment. Equal letters do not differ statistically from each other, to 5% probability of error by Tukey Test.

Seedlings from isolated zygotic embryos such as the seedlings at a concentration of 2 mg L^{-1} GA_3 showed better development (Figure 2e). It was also verified that the seedlings cultivated in the medium containing GA_3 at a concentration of 4 mg L^{-1} (Figures 2c and 2f) had a shorter length when compared to the others, showing curled leaves and aerial part deformation, characterizing toxicity.

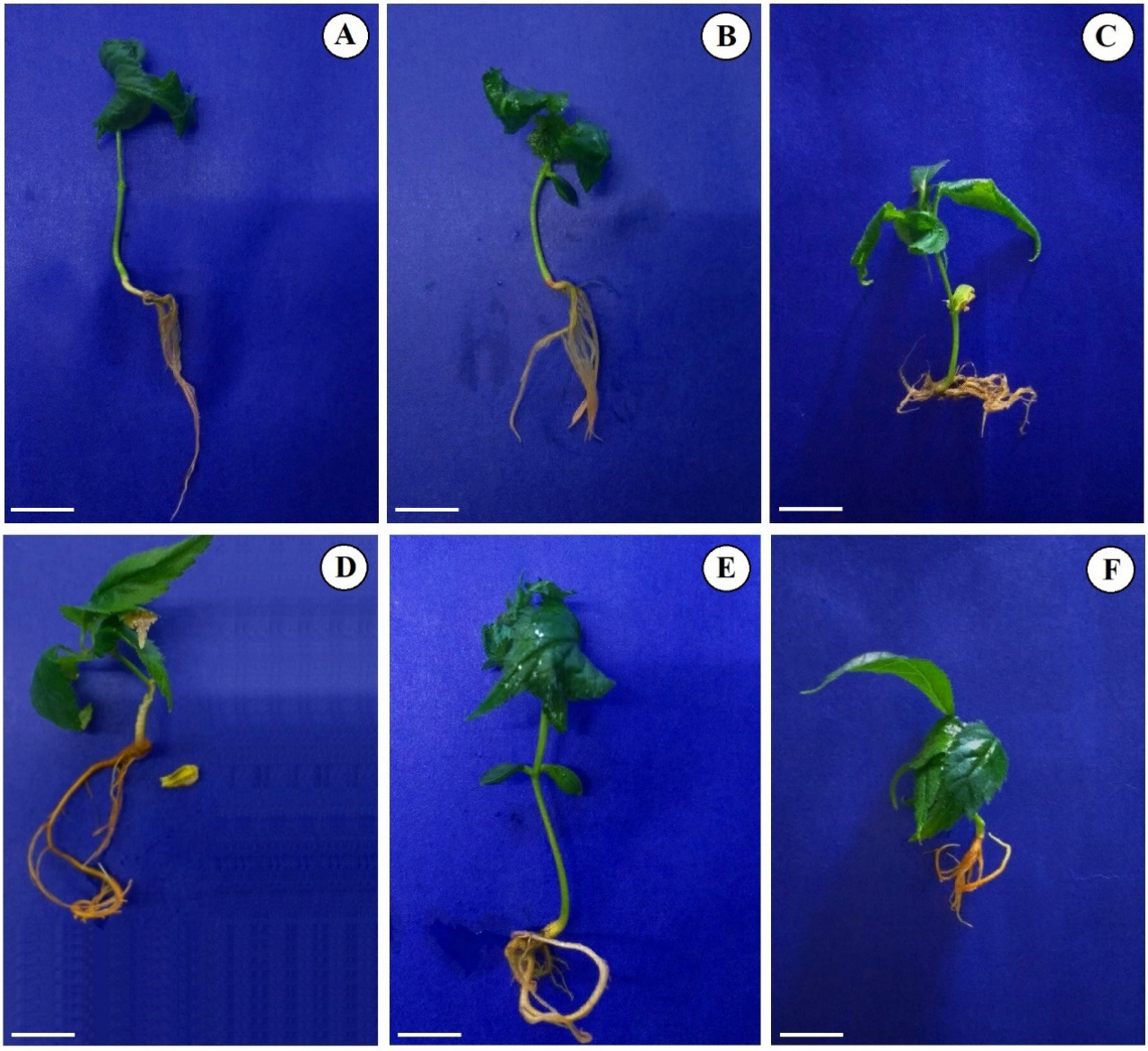

Figure 2 – Seedlings of *Prunus campanulata* at 32 days of *in vitro* incubation. Seedling from seeds without endocarp: (A) 0 mg.L^{-1} GA_3; (B) 2 mg.L^{-1} GA_3; (C) 4 mg.L^{-1} de GA_3; Seedling from isolated zygotic embryos: (D) 0 mg.L^{-1} de GA_3; (E) 2 mg.L^{-1} de GA_3; (F) 4 mg.L^{-1} de GA_3. Scale bars = 10 mm

4 DISCUSSION

Since no germination was observed for complete seeds, the results herein suggested the presence of mechanical dormancy due to the stony and woody endocarp of *P. campanulata*. This characteristic is corroborated by the seed processing treatments, i.e. seeds without tegument and isolated zygotic embryos, as germination was greater than 50% for both treatments, although no statistical differences were observed between these treatments.

The dormancy of *P. campanulata* seeds is assigned to endocarp influences associated with hormonal balance[8]. However, the presence of an endocarp does not seem to be limiting[26], as this structure is permeable when mature[8]. The same is valid for *P. yedoensis*, in which germination is limited by hormonal balance and not by physical obstruction caused by the seed coat[9]. Moreover, according to Fowler and Bianchett[27] mechanical dormant seeds do not germinate even under favorable conditions, demanding dormancy break treatments, such as scarification, stratification, water immersion, or complete endocarp and tegument removal. In this way, the authors also highlighted that when the zygotic embryo is isolated the germination is observed, as well as observed for *P. campanulata* in this study.

Regarding the regulation of hormonal balance in seed dormancy, Lee et al.[10] observed protein expression variations in seeds of *P. campanulata* after stratification (applying high and low temperatures), and as the protein expression changed the hormonal balance changed as well, causing abscisic acid (ABA) reduction up to 12 times and an increase of gibberellins in the seeds, influencing dormancy regulation[17]. Thus, hormonal changes, as well as exogenous gibberellin application, can remove the chemical barrier that limits radicle development, similarly to the stratification technique[8]. Besides that, Chen et al.[8] identified that ABA contents are greater in the endocarp in comparison to the seed coat and the embryo, moreover, when the seed endocarp was removed, the seed dormancy was removed. Finally, dormancy can be genetically controlled in the seeds, so this character can be explored in breeding programs, such as for *P. persica* (L.) Batsch[17].

Dormancy on *P. campanulata* can be associated with combined mechanical resistance and hormonal balance in the tegument since Chen et al.[8] observed that when the endocarp is removed, the dormancy is suppressed. On the other hand, it is important to highlight that seed imbibition in water, such as in this study for seeds with seed coat and isolated zygotic embryos, can contribute to germination, once seed wash ensures chemical dormancy break on seed layers[27].

In addition, the results of this study also suggest dormancy caused by the tegument, since the application of exogenous gibberellin does not enhance germination, even though gibberellins act in seed development[28] and activates DNA of aleurone cells for α-amylase production[29]. Moreover, it was observed an increase in seed germination after seed processing treatments. The use of sodium hypochlorite in the disinfection process may be related to reductions in seed germination, since the salt changes gibberellin and abscisic acid ratio reducing active gibberellin levels, impairing seed germination[30], which is reduced by GA3 treatment[31].

According to Chen et al.[8] *P. campanulata* dormancy is not physiologic, as endocarp remotion

promoted germination rate and time reduction. In contrast, in this study seeds without endocarp had the same germination rate as isolated zygotic embryos[8]. Similar results were observed for zygotic embryos of *Byrsonima cidoniifolia* A. Juss. incubated *in vitro*, being low germination rate is attributed to limitations induced by the endocarp and dormancy, thus the use of zygotic embryos *in vitro* can contribute to reducing germination time and promote the production of viable seedlings without contaminants[32].

Considering the techniques to promote dormancy break, although stratification increases *P. campanulata* germination rate, this technique demands a long period, up to 14 weeks[10] or even 18 weeks[8]. In this context, removing the endocarp and exogenous application of GA_3 can be viable alternatives to obtain seedlings since these techniques can improve germination rates[9]. Furthermore, when *P. campanulata* endocarp is removed or the embryo is isolated, it allows a germination time ranging from six to eight days, similar to seven to 13 days for stratified seeds as observed by Chen et al.[8], evidencing the advantage of the seed processing treatments used in this study.

The germination percentage in this study was lower than observed by Lee et al.[10] ranging from 72% (12 weeks) to 98% (14 weeks) for stratified seeds, and by Chen et al.[8] of 100% and 25% for isolated zygotic embryos and seeds without endocarp, respectively, after 21 days. Despite that, the simple seed imbibition and endocarp remotion in addition to *in vitro* incubation substantially improved germination rate and time, as germination was observed three days after incubation originating normal seedlings appropriate for ex vitro adaptation in less than 30 days. Germination was not induced by the different GA_3 concentrations tested, contrasting the effects observed by Grisez et al.[26], reporting that gibberellin is effective in promoting germination after endocarp remotion. Similarly, Chen et al.[8] highlighted that the phytoregulator is partially effective on unstratified intact seeds, however, when the endocarp is removed, GA_3 application can promote germination. On the other hand, Kim[9] reported the benefic effects of GA_3 on the germination of intact *P. yedoensis* seeds, reaching a rate of 71%, suggesting that dormancy is associated with the hormonal ratio in the seeds. Finally, exogenous gibberellin application can contribute to the germination uniformity of *Prunus* species.

Development impairment by high GA_3 concentrations was reported herein for zygotic embryos of other woody species incubated *in vitro*[33-34]. In this context, Mendes et al.[11] highlighted that high concentrations of gibberellins can result in toxicity limiting seedling development. High concentrations can lead to anomalies as enzyme activity increases in such situations, causing cell wall degradation, corroborating the results observed in this study. In this research, although GA3 did not promote significant effects on germination, the phytoregulator induced biometric changes, improving aerial part and root growth and leaf formation. However, among concentrations was observed growth impairment when the gibberellin concentration was 4 mg L^{-1}, in comparison to the seeds incubated without GA_3 (Figure 2), suggesting that high concentrations cause the plant to be known as reported by Mendes et al.[11]

5 CONCLUSION

In conclusion, the germination only of seeds without endocarp and isolated zygotic embryos confirmed the presence of mechanical dormancy of *P. campanulata*, which was not influenced by the GA_3 concentrations tested. On the other hand, endocarp remotion in addition to *in vitro* incubation promoted a greater germination rate, whereas GA_3 promoted greater seedling growth at concentrations between 1.89 mg L^{-1} for a total length of isolated zygotic embryo and 2.24 mg L^{-1} for the length of aerial part of seeds without endocarp.

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