

Culture media and bap concentrations in the embryo culture of ‘BRS Kampai’ peach

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Abstract -Early-ripening peach cultivars present difficulties in the process of natural germination of seeds. In order to solve this problem, the culture of embryos *in vitro* can be considered an alternative, in which seeds find conditions to complete germination and development in a satisfactory way. Different protocols and culture media have been tested to meet the nutritional needs of the embryo, but they still have problems and need to be optimized. The objective of this work was to test culture media and BAP concentrations in order to increase the germination percentage of embryos and viable ‘BRS Kampai’ peach seedlings, avoiding possible anomalies in the process. The experiment was carried out at the Laboratory of Tissue Culture – “Universidade Tecnológica Federal do Paraná”, *Campus* of Pato Branco. The experimental design used was randomized blocks, in 2x5 factorial, with four replicates, with plot being represented by 15 embryos. The culture media tested were MS and WPM with five BAP concentrations (0, 1, 2, 3 and 4 mg L⁻¹). In the *in vitro* period, seed germination was evaluated through the attribution of scores, and embryo development, measuring the length of stem and main root. During the acclimatization period, seedlings were evaluated for survival, stem length, viable seedlings and rosette formation. Considering the conditions under which the experiment was conducted, it was concluded that MS culture medium with the addition of 1 mg L⁻¹ of BAP allowed higher germination percentage and viable ‘BRS Kampai’ peach seedlings.

Index terms: Embryo Cultivation. *In vitro* cultivation. Cytokine. *Prunus persica*.

Meios de cultura e concentrações de bap na embriocultura do pessegueiro ‘BRS Kampai’

Resumo - As cultivares de pêsego com maturação precoce apresentam dificuldades no processo de germinação natural das sementes. Para solucionar esse problema, a cultura de embriões *in vitro* pode ser considerada uma alternativa na qual as sementes encontram condições para completar a germinação e desenvolvimento de maneira satisfatória. Diferentes protocolos e meios de cultura foram testados para atender às necessidades nutricionais do embrião, mas estes ainda apresentam problemas e precisam ser otimizados. O objetivo do trabalho foi testar meios de cultura e concentrações de BAP a fim de aumentar a porcentagem de germinação de embriões e mudas viáveis de pessegueiro ‘BRS Kampai’, evitando possíveis anomalias no processo. O experimento foi realizado no Laboratório de Cultura de Tecidos da Universidade Tecnológica Federal do Paraná, *Campus* Pato Branco. O delineamento experimental utilizado foi blocos casualizados, em fatorial 2x5, com quatro repetições, sendo a parcela representada por 15 embriões. Os meios de cultura testados foram MS e WPM com cinco concentrações de BAP (0, 1, 2, 3 e 4 mg L⁻¹). No período *in vitro*, foi avaliada a germinação das sementes, através da atribuição de notas, e desenvolvimento dos embriões, mensurando-se os comprimentos de caulículo e radícula principal. No período de aclimatização, as plântulas foram avaliadas quanto à sobrevivência, comprimento do caule, plântulas viáveis e formação de rosetas. Considerando as condições sob as quais o experimento foi conduzido, concluiu-se que o meio de cultura MS com a adição de 1 mg L⁻¹ de BAP permitiu uma maior porcentagem de germinação e plântulas viáveis para ‘BRS Kampai’.

Termos para indexação: Cultivo de Embriões. Cultivo *in vitro*. Citocinina. *Prunus persica*.

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Introduction

'BRS Kampai' peach tree (RASEIRA et al., 2010) is a cultivar launched by "Embrapa Clima Temperado" stone fruit breeding program in Pelotas, which has been widely cultivated and has shown great adaptability and productive stability (SCARIOTTO et al., 2013; CITADIN et al., 2014). However, it appears that seeds of early-ripening peach cultivars, such as 'BRS Kampai', do not germinate due to the immaturity condition of the embryo (SUNDOURI, 2014) and to the accelerated growth and ripening of fruits that does not allow time for the embryo to reach the ideal size and physiological maturation (RAMMING et al., 2003). The rapid hardening of the stone reduces the accumulation of organic matter in the seed, causing abnormality in embryo development, preventing germination from occurring under natural conditions. This phenomenon hinders the formation of seedlings in sufficient volume, hindering the continuity of genetic breeding programs for peach trees (BARBOSA et al., 1985).

As the use for reproductive purposes is hampered by the low or null seed germination, it is necessary to rescue embryos *in vitro* (SUNDOURI, 2014). Embryo culture provides ideal conditions for the immature zygotic embryo to complete its development cycle in aseptic medium (DEVI et al., 2017). Several nutritional media have been used in the *in vitro* rescue of *Prunus* embryos, most of which based on MS medium and its variations (GERCHEVA et al., 2002; LIU et al., 2007; REIS et al., 2012; SUNDOURI et al., 2014; MANSVELT et al., 2015; DEVI et al., 2017) and WPM (RAMMING et al., 2003; RASEIRA; EINHARDT, 2010; NASCIMENTO et al., 2017).

In the *in vitro* cultivation of plant species, the type of medium and concentrations of growth regulators, especially cytokinin, are determining factors to achieve favorable results (SILVEIRA et al. 2001). The cytokinin most used for this purpose is BAP (6-benzylaminopurine) (VILLA et al., 2006) due to its efficiency in the multiplication of shoots (HU; WANG, 1983).

The use of embryo culture for species of the genus *Prunus* can present some difficulties. Some genotypes may show disturbances in their physiology or morphology (RADMANN, 2009), such as rosette formation. This anomaly arises from growth inhibitors located in the apical meristems of embryos (BARBOSA et al., 1989) and their formation is dependent on the cultivar (REIS et al., 2012).

In this context, the main aim of this work was to test culture media with the addition of different BAP concentrations aiming to increase the germination percentage of embryos and viable 'BRS Kampai' peach seedlings, avoiding process anomalies.

Material and methods

The experiment was carried out at the Laboratory of Tissue Culture of the "Universidade Tecnológica Federal do Paraná (UTFPR)" - *Campus* of Pato Branco. In the experiment, early-ripening 'BRS Kampai' seeds with flowering to fruiting period of 95 days were used. Fruits obtained by free pollination were harvested from plants kept in the UTFPR experimental area, in Pato Branco, at the beginning of maturation, when the background color changed from green to cream. Immediately, fruits were transferred to the laboratory and immersed in 10 liters of water containing 10 mL of liquid detergent for 1 minute. Subsequently, samples were submitted to treatment in 0.5% sodium hypochlorite solution for 10 minutes and, subsequently, in 70% alcohol solution for 5 minutes for disinfection (REIS et al., 2012).

In a laminar flow chamber with aseptic conditions, pulp and endocarp were cut without injuring the embryo using previously flamed pruning scissors. With tweezers, also flamed, seeds were extracted. Carefully, when removing the seed, its seed coat was extracted, with immediate and individual inoculation in autoclaved test tubes in contact with different culture media and 6-benzylaminopurine (BAP) concentrations.

The culture media used were MS - Murashige-Skoog (MURASHIGE; SKOOG, 1962) and WPM - Wood Plant Media (LLOYD et al., 1980), according to protocol used by Reis et al. (2012), both supplemented with 30 g L⁻¹ of sucrose, 0.5 mg L⁻¹ of indolbutyric acid (AIB), and 7 g L⁻¹ of agar with pH adjusted to 5.8. BAP concentrations varied according to treatment of 0, 1, 2, 3 or 4 mg L⁻¹. The experimental design was randomized blocks in a 2x5 factorial scheme (culture medium x BAP concentration), with four replicates, with plot being represented by 15 embryos.

Subsequently, test tubes were closed with aluminum foil, sealed, identified, placed in paper box and placed in BOD (Biochemical Oxygen Demand) chamber at constant temperature of 5 ± 1°C in the dark for sixty days for seed stratification. After this period, tubes were maintained at temperature of 24 ± 1°C, remaining for two days in the dark and seven days exposed to light at 2,000 lux of intensity for chlorophyll formation, also at 24 ± 1°C with 16-hour photoperiod (REIS et al., 2012).

Seeds were evaluated for beginning of germination according to the scale of scores (REIS et al., 2012): 1 - absence of germination; 2 - abnormal germination only with open cotyledons; 3 - abnormal germination only with root emission; 4 - abnormal germination only with stem emission; 5 - normal germination with stem and primary root at two and nine days after removal from the cold chamber. The percentage of normal germination was also determined, taking into account only embryos that scored 5 in the second count.

For the evaluation of embryo development after nine days of exposure in growth chamber, seedlings were carefully removed from test tubes, washed in running water to remove residues of the culture medium adhered to roots and placed in plastic trays with paper towels moistened with distilled water to avoid dehydration. Stem and root length was measured using millimeter ruler (REIS et al, 2012).

After stem and main root measurements, all seedlings, even those in abnormal condition, were transferred to tubes containing sterilized commercial substrate and placed in Fitotron growth chamber for acclimatization at temperature of 24 °C and 95% humidity. Hoagland and Arnon's nutrient solution was applied weekly (1938).

At 60 days after planting, survival percentage, viable seedlings, leaf rosette formation and stem length were evaluated.

To calculate seedling survival percentages, the initial number of transplanted seedlings was considered. For the percentage of viable seedlings and rosettes, the final number of survivors 60 days after transplantation was considered.

Data were initially submitted to the Shapiro-Wilk test to verify the normality of errors and the Oneillmathews test to verify the homogeneity of variances. Significant variables by the F test were submitted to the Tukey average comparison test. Main root and stem length, viable seedlings and leaf rosette formation variables did not meet mathematical assumptions, even after transformation, and were submitted to the Friedman test ($p = 0.05$). All statistical analyses, as well as figures, were performed in R language (R CORE TEAM, 2018).

Results and Discussion

In the *in vitro* embryo culture, there was significant interaction only for the normal germination variable, while for embryo germination and stem and root length variables, no significance was observed.

In the first embryo germination evaluation carried out two days after removal from the cold chamber, both culture media had higher percentages of scores 2, indicating that embryos presented abnormal germination only with open cotyledons, and in WPM medium, this condition was superior compared to the MS medium (Figure 1).

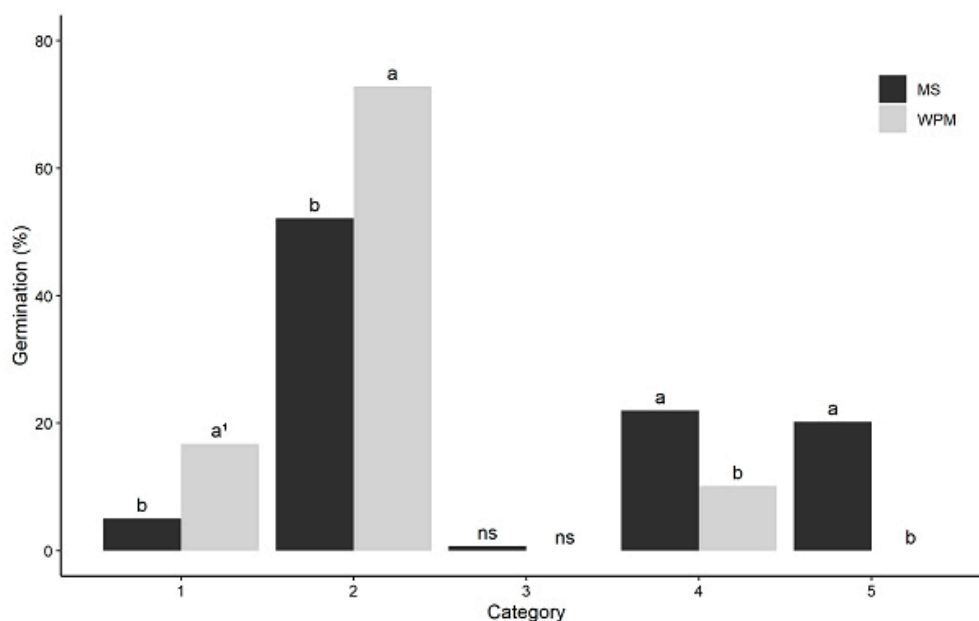


Figure 1. Germination score two days after removal from the cold chamber of 'BRS Kampai' peach embryos in relation to the type of culture medium. UTFPR, Campus of Pato Branco, 2020. ¹ Means followed by different letters in the column differ from each other by the Tukey test ($p \leq 0.05$). ^{ns} Not significant. 1) Absence of germination; 2) Abnormal germination only with open cotyledons; 3) Abnormal germination only with root emission; 4) Abnormal germination only with stem emission and 5) Normal germination with stem and root. MS - Murashige-Skoog culture medium (MURASHIGE; SKOOG, 1962). WPM - Wood Plant Media (LLOYD et al., 1980)

In the second evaluation carried out nine days after the removal of embryos from the cold chamber, it was found that the MS medium had the highest percentage of scores 5, indicating that embryos had normal germination with the presence of well-developed root and stem, while the WPM medium presented, as in the first evaluation, predominance of embryos only with open cotyledons (Figure 2). No absence of seed germination in this evaluation was observed.

For the percentage of normal germination, considering only embryos that scored 5 in the second evaluation, there was significant interaction between types of medium and BAP concentrations, that is, the behavior of the culture media in relation to the normal germination of embryos changed whether or not adding BAP. The addition of 1 mg L⁻¹ of BAP to the MS medium showed the highest percentage of embryo germination under normal conditions (Table 1).

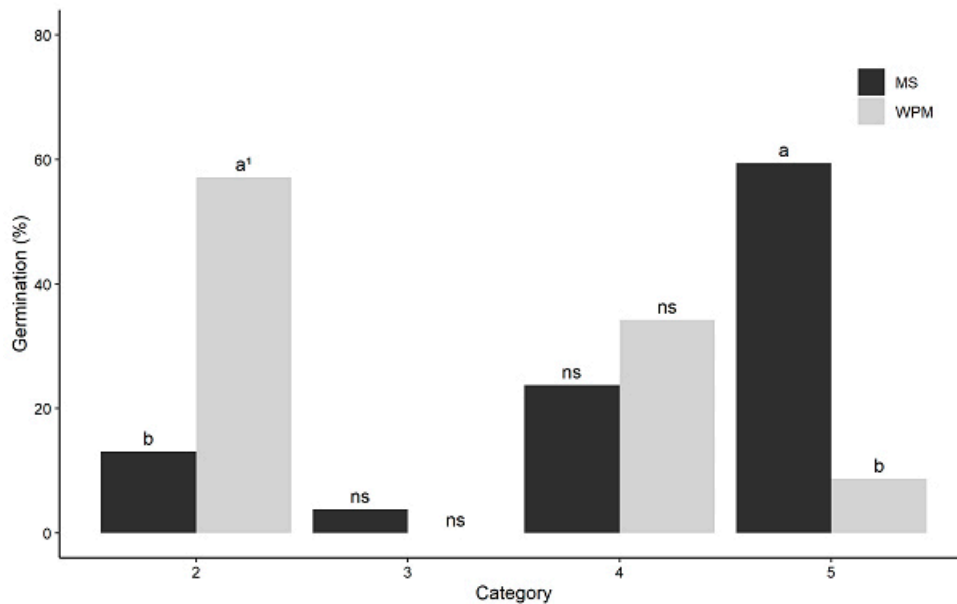


Figure 2- Germination score nine days after removal from the cold chamber of ‘BRS Kampai’ peach embryos in relation to the type of culture medium and BAP concentration. UTFPR, Campus of Pato Branco, 2020. ¹ Means followed by different letters in the column differ from each other by the Tukey test ($p \leq 0.05$). ^{ns} Not significant. 1) Absence of germination; 2) Abnormal germination only with open cotyledons; 3) Abnormal germination only with root emission; 4) Abnormal germination only with stem emission and 5) Normal germination with stem and root. MS - Murashige-Skoog culture medium (MURASHIGE; SKOOG, 1962). WPM - Wood Plant Media (LLOYD et al., 1980).

Table 1 - Normal germination of ‘BRS Kampai’ peach embryos as a function of five BAP concentrations (0, 1, 2, 3 and 4 mg L⁻¹) and two culture media (MS and WPM). MS - Murashige-Skoog culture medium (MURASHIGE; SKOOG, 1962). WPM - Wood Plant Media culture medium (LLOYD et al., 1980). UTFPR Campus of Pato Branco, 2020

Culture media	BAP (mg L ⁻¹)				
	0	1	2	3	4
MS*	63.34 Aab ¹	78.33 Aa	55.55 Ab	55.56 Ab	44.45 Ab
WPM**	43.33 Ba	0.00 Bb	0.00 Bb	0.00 Bb	0.00 Bb
CV (%)	30.52				

¹Means followed by uppercase letters in the column and lowercase letters in the row differ significantly by the F test and Tukey test ($p \leq 0.05$), respectively. * MS - Murashige-Skoog culture medium (MURASHIGE; SKOOG, 1962). ** WPM - Wood Plant Media (LLOYD et al., 1980).

For the main root and stem development, the MS medium allowed the highest averages for both variables (Table 2). Regarding the BAP concentration, the use of MS medium added of 1 mg L⁻¹ of BAP allowed greater main root development, while the use of this growth regulator at concentrations between 1 and 4 mg L⁻¹ in the WPM medium was harmful (Table 3). For stem development

in the MS medium, the use of 4 mg L⁻¹ of BAP provided the greatest seedling length, not differing from 1 mg L⁻¹, whereas in the WPM medium, the absence of BAP or the use of 1 mg L⁻¹ favored the highest stem development (Table 4).

Table 2 - Averages and rankings of the multiple comparisons test of Friedman ($\alpha = 0.05$) for main root and stem length variables of 'BRS Kampai' peach seedlings in relation to type of culture medium. UTFPR Campus of Pato Branco, 2020

Culture media	Main root		Stem	
	Means (cm)	Ranking	Means (cm)	Ranking
MS*	1.30	8.00 a ¹	2.26	8.00 a ¹
WPM**	0.11	4.00 b	0.36	4.00 b

¹Means followed by distinct letters in the vertical differ from each other by the Friedman test ($\alpha = 0.05$). * MS - Murashige-Skoog culture medium (MURASHIGE; SKOOG, 1962). ** WPM - Wood Plant Media (LLOYD et al., 1980).

Table 3. Averages and rankings of the multiple comparisons test of Friedman ($\alpha = 0.05$) for main root and stem length variables of 'BRS Kampai' peach seedlings in relation to type of culture medium. UTFPR Campus of Pato Branco, 2020

BAP (mg L ⁻¹)	Main root			
	MS*		WPM**	
	Means (cm)	Ranking	Means (cm)	Ranking
0	1.77	16.00 b ¹	0.42	20.00 a
1	3.04	20.00 a	0.05	11.50 c
2	0.60	8.00 d	0.00	4.00 d
3	0.35	4.00 e	0.04	9.50 c
4	0.76	12.00 c	0.07	15.00 b

¹Means followed by distinct letters in the vertical differ from each other by the Friedman test ($\alpha = 0.05$). * MS - Murashige-Skoog culture medium (MURASHIGE; SKOOG, 1962). ** WPM - Wood Plant Media (LLOYD et al., 1980).

Table 4. Averages and rankings of the multiple comparisons test of Friedman ($\alpha = 0.05$) for stem length variable of 'BRS Kampai' peach in relation to BAP concentration. UTFPR Campus of Pato Branco, 2020

BAP(mg L ⁻¹)	Stem length			
	MS*		WPM**	
	Means (cm)	Ranking	Means (cm)	Ranking
0	2.37	13.00 b ¹	0.49	19.00 a
1	2.53	16.00 ab	0.45	15.00 ab
2	1.81	8.00 c	0.29	9.00 c
3	1.63	4.00 d	0.37	13.00 bc
4	2.95	19.00 a	0.22	4.00 d

¹Means followed by distinct letters in the vertical differ from each other by the Friedman test ($\alpha = 0.05$). * MS - Murashige-Skoog culture medium (MURASHIGE; SKOOG, 1962). ** WPM - Wood Plant Media (LLOYD et al., 1980).

Regarding seedling acclimatization, no significant interaction between type of medium and BAP concentrations was observed for any of the variables under study. For the survival percentage of seedling at 60 days after planting, no significant difference was observed for types of media, only for BAP concentrations. For both culture media, the seedling survival behavior is represented by a decreasing linear equation, indicating that the increase in BAP concentration in the culture medium causes reduction in the survival of peach seedlings at 60 days after transplantation (Figures 3 A, B).

As for the percentage of viable seedlings and rosettes, no significant difference was observed between culture media. Regarding BAP concentration, it was observed that the absence of the regulator or use of regulator at concentration greater than 3 mg L⁻¹ in the MS medium caused reduction in the percentage of viable seedlings and, consequently, increase in the occurrence of leaf rosette formation at other concentrations (Table 5). In WPM medium, it was observed that the use of BAP concentrations of 1 and 2 mg L⁻¹ provided higher percentage of viable seedlings and lower occurrence of rosettes.

Regarding stem growth, significant difference was only observed for types of medium, with MS medium providing higher stem growth compared to the WPM medium (Table 6).

Table 5. Means and rankings of the multiple comparisons of Friedman test ($\alpha = 0.05$) for viable seedlings and rosettes variables of 'BRS Kampai' peach at 60 days after planting in relation to BAP concentration. UTFPR, Campus of Pato Branco, 2020

BAP (mg L ⁻¹)	MS*			
	Viable seedlings		Rosettes	
	Means (cm)	Ranking	Means (cm)	Ranking
0	86.70	6.00 b ¹	13.30	18.00 b
1	100.00	13.50 a	0.00	10.50 a
2	100.00	13.50 a	0.00	10.50 a
3	86.70	6.00 b	13.30	18.00 b
4	86.70	6.00 b	13.30	18.00 b
BAP (mg L ⁻¹)	WPM**			
	Viable seedlings		Rosettes	
	Means (cm)	Ranking	Means (cm)	Ranking
0	73.23	13.00 b ¹	26.77	11.00 b
1	92.26	18.50 a	7.74	5.00 c
2	90.87	16.50 ab	9.13	8.00 cb
3	47.50	7.50 c	52.50	16.50 a
4	33.33	4.50 c	66.67	19.50 a

¹Means followed by distinct letters in the vertical differ from each other by the Friedman test ($\alpha = 0.05$). * MS - Murashige-Skoog culture medium (MURASHIGE; SKOOG, 1962). ** WPM - Wood Plant Media (LLOYD et al., 1980).

Table 6. Stem length of 'BRS Kampai' peach after 60 days in Fitotron chamber in relation to type of culture medium and BAP concentration. UTFPR Campus of Pato Branco, 2020

Culture medium	Stem (cm)
MS*	2.99 a ¹
WPM**	1.55 b
CV (%)	35.71

*Means followed by different letters differ significantly by the F test ($p \leq 0.05$). * MS - Murashige-Skoog culture medium (MURASHIGE; SKOOG, 1962). ** WPM - Wood Plant Media culture medium (LLOYD et al., 1980).

The MS medium is efficient for the germination of *Prunus* embryos, confirmed by several studies (GERCHEVA et al., 2002; LIU et al., 2007; REIS et al., 2012), which obtained the highest germination percentages when this type of medium was used.

In the present study, the use of the MS medium with the addition of 1 mg L⁻¹ of BAP showed the highest embryo germination percentage under normal conditions, that is, those with developed shoot and root system, while the highest BAP concentrations (2, 3 and 4 mg L⁻¹) resulted in the formation of abnormal embryos (Table 1). Similar results were found in the rescue of 'Brumosa' plum embryos and the best results for embryo germination and development were achieved when 0.70 mg L⁻¹ of BAP was added to the culture medium (GERCHEVA et al., 2002). However, these results do not corroborate those observed for the production of intraspecific hybrids of 'Zhonghuashoutao' peach crossed with 'Morettini' plum, since higher BAP concentrations (4.0 mg L⁻¹) promoted 90% of embryo germination (LIU et al., 2007). The contradictions expressed above show that the efficiency of the use of growth regulators with cytokine effect depends

on the species, cultivar and medium used (TAIZ; ZEIGER, 2004), always requiring tests for adjustments of protocol and definitions of concentrations to be used, depending on each case.

In both culture media, high BAP concentrations seemed to be detrimental to normal embryo germination (Table 1). Balance, interaction and endogenous phytohormone concentration of the explant determine the *in vitro* morphogenetic process. Although auxins and cytokinins are normally required for growth or morphogenesis, auxins can inhibit cytokinin accumulation, while the latter can inhibit at least some actions of auxins (MONFORT et al., 2012).

In this case, the addition of BAP may have restricted the activity of auxins. The non-formation of roots may explain the lack of normal germination (Table 1) when BAP was added to the WPM medium, showing that this regulator has negative effect on this factor. Interactions between cytokinin and auxin are fundamental in the control of plant morphogenesis, and the *in vitro* exogenous application of these two classes of plant regulators generates antagonistic effects (SKOOG;

MILLER, 1957). In addition, the addition of BAP usually inhibits or delays the formation of roots (BEN-JAACOV et al., 1991).

The WPM medium showed low efficiency compared to the MS medium, both for germination of embryos (Figures 1 and 2) and for the development of peach seedlings (Table 2). Such results corroborate Reis et al. (2012) and Fermino Junior and Scherwinski-Pereira (2012), who tested different culture media for the germination of peach and cherry seeds, respectively, and obtained the worst results with WPM. What may explain the inefficiency of this medium for the rescue of peach embryos is the low concentration of salts compared to the MS medium, especially NO_3^- and NH_4^+ (VILLA et al., 2006).

Both culture media used presented median survival percentage (Figure 3); however, when there was no addition of BAP, survival was greater in relation to treatments in which BAP concentrations were added, in which the percentage of surviving seedlings reduced gradually. The evidence that the presence of BAP impaired survival at 60 days after transplantation leads to the hypothesis of supposed phytotoxicity induced by high dosages of the growth regulator. Future experiments should test lower BAP concentrations, between 0 and 1 mg L^{-1} , because for higher concentrations, there are positive responses in obtaining normal seedlings using the MS medium.

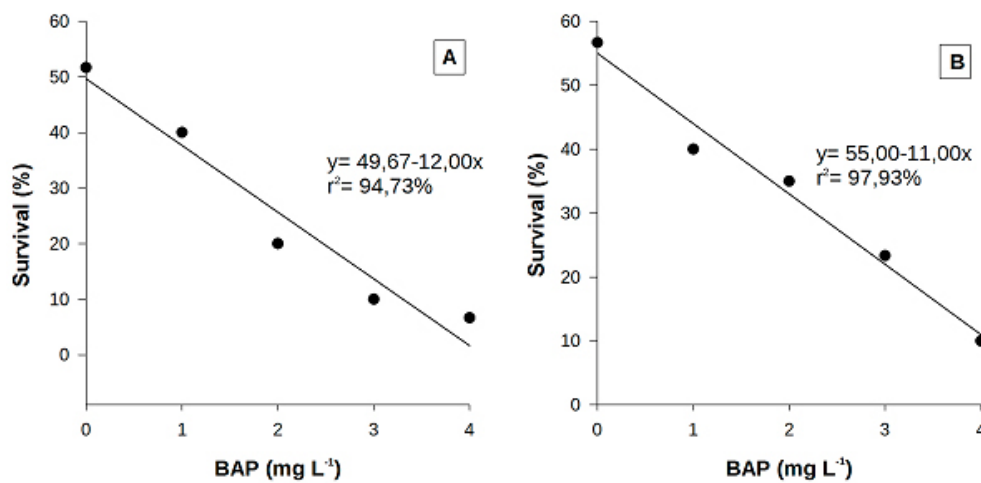


Figure 3 - Survival of 'BRS Kampai' peach seedlings at 60 days after planting in MS (A) and WPM (B) culture media as a function of five BAP concentrations (0, 1, 2, 3 and 4 mg L^{-1}). UTFPR Campus of Pato Branco, 2020.

Although seedling survival was higher in the absence of BAP in both MS and WPM media, the percentage of viable seedlings did not follow the same trend. In the acclimatization period, the use of BAP at low concentrations allowed increases in the percentage of seedlings in development conditions, reducing and / or nullifying the presence of leaf rosettes (Table 5). Rosette formation is classified as a disorder that affects leaf morphology, causing asymmetry and twisting due to reduced growth. In this case, leaves aggregate small, compact cells without chlorophyll, resulting in abnormal seedlings (FLEMION; BEARDOW, 1964).

In treatments without addition of BAP to the medium, the occurrence of the anomaly increased (Table 5). This result corroborates Reis et al. (2012), since peach seedlings grown in MS and WPM media without the addition of growth regulators presented 11.42% and 24.99% of rosettes, respectively. Thus, even if there is reduction in survival with the addition of 1 mg L^{-1} of BAP to the media (Figure 3), this reduction is compensated by the increase in viable seedlings (Table 5), especially when using MS medium.

Prolonged exposure to BAP at concentrations of 3 and 4 mg L^{-1} may have caused the formation of rosettes (ANDRADE, 2011). Higher BAP concentrations (3.4 mg L^{-1} and 4.5 mg L^{-1}) promoted symptoms of leaf rosettes in early peach and nectarine seedlings (BARBOSA et al., 1989).

In addition to the influence of BAP, the occurrence of rosettes in peach seedlings may have been favored by the association with other factors, among which are temperature, stratification duration and cultivar response. In the subtropics, peach seeds are harvested in late spring, stratified and germinated in summer, when maximum temperatures are generally above 30 °C, which is similar to the conditions of this experiment, but rosette formation can be a great problem under these conditions (TOPP et al., 2008). The formation of rosettes is also dependent on the cultivar and it was found to be high in seeds of genotypes with fruit development period of less than 110 days, as is the case of 'BRS Kampai', with a 95-day cycle, decreasing rapidly with increase of the fruit development period (BACON; BYRNE, 1995).

The results obtained in the present study may indicate that the 'BRS Kampai' genotype has difficulty in germinating seeds and is dependent on a culture medium that provides higher concentration of salts, such as MS, supplemented with low auxin and cytokinin levels. Some cultivars may have differential biosynthesis of endogenous hormones and / or different efficiency in the way of absorbing and metabolizing compounds present in the culture medium (PARFITT; ALMEHDI, 1986). Thus, obtaining favorable results depends on several factors, mainly those related to the behavior of each material due to the interaction between genotype and culture medium (CABETAS et al., 1997).

Conclusion

MS medium supplemented with 1 mg L⁻¹ of BAP is recommended for the embryo culture of 'BRS Kampai' peach.

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References

ANDRADE, G.R. **Resposta do sistema antioxidativo sob a influência de 6-benzilaminopurina na micropropagação de cana-de-açúcar**. 2011. 86 f. Dissertação (Mestrado em Química) - Universidade Federal Rural de Pernambuco, Recife, 2011.

BACON, T.A.; BYRNE, D.H. Relationships of fruit development period, seed germination, seedling survival and percent dry weight of ovule in peach. **HortScience**, Alexandria, v.30, n.4, p.833, 1995.

BARBOSA, W.; DALL'ORTO, F.A.C.; OJIMA, M. Eliminação de anomalias fisiológicas, in vitro, de plântulas de pessegueiro. **Bragantia**, Campinas, v.48, n.1, p.13-19, 1989.

BARBOSA, W.; DALL'ORTO, F.A.C; OJIMA, M. Cultura de embriões in vitro para o melhoramento de pessegueiros precoces. **Bragantia**, Campinas, v.44, n.1, p.465-472, 1985.

BEN-JAACOV, J.; ACKERMAN, A.; TAL, E.; JACOBS, G. Vegetative propagation of *Albertya magna* by tissue culture and grafting. **HortScience**, Alexandria, v.26, n.2, p.74-75, 1991.

CABETAS, M.J.R.; COMPANY, R.S.I.; MORALES, M.C. Cultivos de óvulos y embriones en programas de mejora genética de frutales. **Fruticultura Profesional**, Barcelona, v.84, n.1, p.58-64, 1997.

CITADIN, I.; SCARIOTTO, S.; SACHET, M.R.; ROSA, F.J.; RASEIRA, M.C.B.; JUNIOR, A.W. Adaptability and stability of fruit set and production of peach trees in a subtropical climate. **Scientia Agricola**, Piracicaba, v.71, n.2, p.133-138, 2014.

DEVI, I.; SINGH, H.; THAKUR, A. Effect of developmental stage and medium on embryo culture of low chill peach hybrids. **Current Science**, Bengaluru, v.113, n.9, p.1771-1775, 2017.

FERMINO JUNIOR, P.C.P.; SCHERWINSKI-PEREIRA, J.E. Germinação e propagação in vitro de cerejeira (*Amburana acreana*). **Ciência Florestal**, Santa Maria, v.22, n.1, p.1-9, 2012.

FLEMION, F.; BEARDOW, J. Histological studies of physiologically dwarfed peach seedlings. I. Structure of anomalous leaves. **Contribution from the Boyce Thompson Institute**, California, v.22, n.1, p.117-131, 1964.

GERCHEVA, P.; ZHIVONDOV, A. Embryo rescue of early ripening plum cultivars. **Acta Horticulturae**, Leuven, v.577, p.165-168, 2002.

HOAGLAND, D.R.; ARNON, D. I. **The water culture method for growing plants without soils**. Berkeley: California Agricultural Experimental Station, 1950. p.347.

HU, C.Y.; WANG, P.J. Meristem, shoot tip and bud culture. In: EVANS, D.A.; SHARP, W.R.; AMMIRATO, P.V.; YAMADA, Y. (ed.). **Handbook of plant cell culture: techniques for propagation and breeding**. New York: Macmillan, 1983. p.117-227.

LIU, W.; CHEN, X.; LIU, G.; LIANG, Q.; HE, T.; FENG, J. Interspecific hybridization of *Prunus persica* with *P. armeniaca* and *P. salicina* using embryo rescue. **Plant Cell, Tissue and Organ Culture**, New York, v.88, n.3, p.289-299, 2007.

- LLOYD, G.; MCCOWN, B. Commercially feasible micropropagation of *Mountain laurel*, *Kalmia latifolia*, by use of shoot-tipe culture. **Proceedings of the International Plant Propagators Society**, Washington, v.30, n.5, p.421-427, 1980.
- MANSVELT, E.L.; PIETERSE, W-M.; SHANGE, S.B.D.; MABIYA, T.C.; CRONTÉ, C.; BALLA, I.; HAM, H.; RUBIO-CABETAS, M.J. Embryo rescue of *Prunus persica*: medium composition has little influence on germination. **Acta Horticulturae**, Leuven, v.1084, n.1084, p.207-210, 2015.
- MONFORT, L.E.F.; PINTO, J.E.B.P.; BERTOLUCCI, S.K.V.; ROSSI, .T.T.; SANTOS, F.M. Efeito do BAP no cultivo in vitro de *Ocimum selloi* Benth. **Revista Brasileira de Plantas Mediciniais**, Botucatu, v.14, n.3, p.458-463, 2012.
- MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. **Physiologia Plantarum**, Copenhagen, v.15, p.473-497, 1962.
- NASCIMENTO, D.C.; DINI, M.; CARPENEDO, S.; RASEIRA, M.C.B. Germinação e desenvolvimento de embriões de pessegueiro 'Precocinho': a ssepsia e uso do PPMTM no meio de cultura. *In*: CONGRESSO BRASILEIRO DE FRUTICULTURA, 25., 2017, Porto Seguro. **Anais [...]** Sociedade Brasileira de Fruticultura, 2017. p.63.
- PARFITT, D.E.; ALMEHDI, A. In vitro propagation of peach: II. A medium for in vitro multiplication of 56 peach cultivars. **Fruit Varieties Journal**, University Park, v.40, n.2, p.46-47, 1986.
- R CORE TEAM. **R**: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2018.
- RADMANN, E.B.; BIANCHI, V.J.; SOUZA, T.M.; FACHINELLO, J.C.; OLIVEIRA, R.P. Influência da composição do meio de cultivo e do tipo de explante na micropropagação do porta-enxerto de *Prunus* sp. 'GxN-9'. **Scientia Agraria**, Curitiba, v.10, n.2, p.95-101, 2009.
- RAMMING, D.W.; EMERSHAD, R.L.; FOSTER, C. *In vitro* factors during ovule culture affect development and conversion of immature peach and nectarine embryos. **HortScience**, Alexandria, v.38, n.3, p.424-428, 2003.
- RASEIRA, M.C.B.; EINHARDT, P.M. Resgate de embriões em pessegueiro: tempo de incubação. **Scientia Agraria**, Curitiba, v.11, n.6, p.445-450, 2010.
- RASEIRA, M.C.B.; NAKASU, B.H.; UENO, B.; SCARANARI, C. Pessegueiro: cultivar BRS Kampai. **Revista Brasileira de Fruticultura**, Jaboticabal, v.32, n.4, p.1275-1278, 2010.
- REIS, L.; CITADIN, I.; PENSO, G.A.; SCARIOTTO, S.; WAGNER JUNIOR, A. Estratificação in vitro de embriões zigóticos de pessegueiro em diferentes meios de cultura e concentrações de sacarose. **Revista Brasileira de Fruticultura**, Jaboticabal, v.34, n.3, p.653-660, 2012.
- SCARIOTTO, S.; CITADIN, I.; RASEIRA, M.C.B.; SACHET, M.R.; PENSO, G.A. Adaptability and stability of 34 peach genotypes for leafing under Brazilian subtropical conditions. **Scientia Horticulturae**, Amsterdam, v.155, p.111-117, 2013.
- SILVEIRA, C.A.P.; FACHINELLO, J.C.; FORTES, G.R.L.; CITADIN, I.; RODRIGUES, A.C.; QUEZADA, A.C.; SILVA, J.B. Multiplicação in vitro de porta-enxertos do gênero *Prunus* sob diferentes concentrações de BAP em dois meios de cultura. **Revista Brasileira de Fruticultura**, Jaboticabal, v.23, n.3, p.488-492, 2001.
- SKOOG, F.; MILLER, C.O. Chemical regulation of growth and organ formation in plant tissue cultures in vitro. **Symposia of the Society for Experimental Biology**, Cambridge, v.11, p.118-131, 1957.
- SUNDOURI, A.S.; SINGH, H.; GILL, M.; THAKUR, A.; SANGWAN, A. In-vitro germination of hybrid embryo rescued from low chill peaches as affected by stratification period and embryo age. **Indian Journal of Horticulture**, Nova Delhi, v.71, n.2, p.151-155, 2014.
- TAIZ, L.; ZEIGER, E. **Fisiologia vegetal**. 3.ed. Porto Alegre: Artmed Editora, 2004. p.719.
- TOPP, B.L.; SHERMAN, W.B.; RASEIRA, M.C.B. Low-chill cultivar development. *In*: LAYNE, D.; BASSI, D. (ed.). **The peach**: botany, production and uses. Wallingford: Cabi International, 2008. p.106-138.
- VILLA, F.; FRAGUAS, C.B.; DUTRA, L.F.; PIO, L.A.S.; PASQUAL, M. Multiplicação in vitro de amoreira-preta cultivar Brazos. **Ciência e Agrotecnologia**, Lavras, v.30, n.2, p.266-270, 2006.