

Original Research Article

Growth regulators on in vitro multiplication of yellow passion fruit

ABSTRACT

Aims: The objective of this work was to evaluate the effect of growth regulators on in vitro multiplication of yellow passion fruit.

Study design: Experimental design was completely randomized in a factorial 5x2 (BAP x presence or absence of NAA) with six replicates, each consisting of a bottle with four explants.

Place and Duration of Study: The study was conducted at Fruit Plant Propagation Laboratory, Federal University of Pelotas, RS. After 60 days of cultivation number of leaves, shoots, average shoot length (cm), average number of roots and average length of roots (cm) were evaluated.

Methodology: Stem segments with a shoot were inoculated on MS medium added or not with growth regulators 6 - benzylaminopurine (BAP) (0.5, 1.0, 1.5, 2.0 and 2.5 mg L⁻¹) and naphthalene acetic acid (NAA) (0 and 0.1 mg L⁻¹), according to the treatment. Then, they were kept in a growth chamber.

Results: The absence of growth regulators promoted higher length of shoots, the largest root and root number. The concentration of 0.5 mg L⁻¹ BAP without the addition of NAA in MS medium showed higher number of leaves and the same dose combined with NAA, promoted the highest number of shoots of *P. edulis* f. *flavicarpa* on in vitro multiplication.

Conclusion: This study developed optimum medium for yellow passion fruit. Different media were observed to be appropriate for the analyzed variables. The procedure can be of great usefulness for mass multiplication and improvement of this economically important plant species.

Keywords: *Passiflora edulis* f. *flavicarpa*; micropropagation; 6 – benzylaminopurine; naphthalene acetic acid; tissue culture.

1. INTRODUCTION

Brazil is the largest passion fruit producer, and also the largest fruit and its derivatives consumer [1]. However, it presents low productivity (about 13 t ha⁻¹ year⁻¹) [2], attributed to its phytosanitary problems, inadequate cultivation techniques and absence of superior cultivars.

The species *Passiflora alata* Curtis, *Passiflora edulis* Sims and *Passiflora edulis* Sims f. *flavicarpa* Degener are the most commercially grown in the country. However, the outstanding species is *Passiflora edulis* f. *flavicarpa*, being responsible for 95% of the Brazilian orchards, due to their desirable agronomic attributes and the excellent ecological conditions for cultivation [3, 4].

In vitro propagation of *Passiflora* genus has been shown to be viable for commercial elite plants production. Plant regeneration system based on organogenesis using 6 - benzylaminopurine (BAP) is currently prevalent in *Passiflora* species, as these processes are more frequent in direct and indirect morphogenesis [5]. The micropropagation technique afford alternatives for greater biomass production and to ensure the economic interest species perpetuation [6].

The addition of growth regulators, especially auxins and cytokinins, play a very important role. The combination of auxin and cytokinin and the isolated use of cytokinin have been very important on in vitro shoots multiplication [7].

Cytokinins play a crucial role in many phases of plant growth and development, and when added to in vitro culture medium, they promote the overcoming of apical dominance by breaking lateral buds dormancy and thus are related to shoot induction [8].

BAP is a commercially available cytokinin, and has been very effective in promoting several species multiplication, being used in approximately 60% of the culture media. The source of cytokinin, as well as its concentration, are the factors that most influence the in vitro development process [9].

Auxins, in turn, are used in culture medium generally for adventitious roots induction. Rooting is a very important phase in micropropagation, due to root involvement in water and nutrients acquisition, among others [10].

According to George and co-authors [8], auxins and cytokinins are the most widely used classes of growth regulators and the balance of both regulators will drive explant development. Auxin and cytokinins may have antagonistic or complementary effects, according to the plantlet development stage [11]. Auxins, being a strong signal to apical dominance, maintains lateral buds dormancy, functioning in the opposite direction to the cytokinins in this case. However, root formation, often induced by auxin addition, can promote shoots growth, since the roots are the main cytokinins sources [12].

The objective of this work was to evaluate the effect of growth regulators (BAP and NAA) on in vitro multiplication of *P. edulis* f. *flavicarpa*.

2. MATERIAL AND METHODS

The experiment was carried out at fruit Trees Propagation Laboratory, Department of Horticulture and Crop Science, Faculty of Agronomy Eliseu Maciel, Federal University of Pelotas, RS, Southern of Brazil, during 2015.

Explants used in this experiment came from yellow passion fruit (*P. edulis* f. *flavicarpa*) already in vitro established, shoots from stem segments and excised apex were used.

Treatments consisted of five BAP concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 mg L⁻¹) and the presence or absence of NAA (0 and 0.1 mg L⁻¹), totaling 10 treatments plus one control factor, without growth regulators addition.

Experimental design was completely randomized in a factorial scheme 5x2 (concentrations of BAP x presence or absence of NAA), with six replicates, each replicate consisting of a flask with four explants.

Explants were inoculated in MS culture medium [13], supplemented with 100 mg L⁻¹ myo-inositol, 30 g L⁻¹ sucrose, with or without regulators, according to the treatments. Culture medium pH was adjusted to 5.8 before agar inclusion at the concentration of 6 g L⁻¹ and then autoclaved at 121°C and 1.5 atm for 20 minutes. Glass flasks with 300 mL capacity were used, with 30 mL of culture medium per flask.

Flasks were sealed with aluminium foil and Parafilm®, and after inoculation, they remained in a growth room at 16h-photoperiod, irradiance of 27 µmol m⁻² s⁻¹ provided by cool white fluorescent lamps, and temperature of 25 ± 2°C.

After 60 days of cultivation, the variables analyzed were number of leaves, average number of shoots, medium shoot length (cm), average number of roots and average length of the longest root (cm).

Atypical values (outliers) were identified with residuals plot externally studentized (RStudent) versus predicted values (variable Y). From the RStudent, outside values from the range -2 to 2 were considered outliers and their corresponding observations were removed from the database [14, 15]. Data were analyzed for normality by the Shapiro-Wilk test; homoscedasticity by Hartley test; and residuals independence by graphical analysis.

Considering the variables, average number of roots and average length of the longest root $\sqrt{(x + 0.5)}$ transformation was necessary. Later, the data were submitted to analysis of variance by F test ($p \leq 0.05$). Observing a statistical significance, the effects of carbohydrate source were compared by Tukey test ($p \leq 0.05$), the DMS test was plotted on the chart and the differences were considered significant when there was no overlap between the vertical bars. The effects of doses were evaluated by polynomial regression models ($p \leq 0.05$), as follows:

$$y = a + bx;$$

meaning: y = variable response; a = maximum estimated value for the variable response; b = slope of the line or curve; x = carbohydrates doses (mM). When there was no equation adjustment, the doses were compared at 95% of confidence intervals, these intervals were plotted on the graph and the differences were considered significant when there was no overlap between vertical bars.

3. RESULTS AND DISCUSSION

It was observed a greater increase in the number of leaves on BAP concentration 0.5 mg L^{-1} and NAA absence (Fig. 1A), but there was no significant difference between the other doses without auxin. It was found that all tested BAP concentrations without NAA differed from the control, indicating the need of the BAP regulator to obtain higher values for this variable. Similar result was obtained by Soares [16], where it was achieved the highest average per explant using 1.0 mg L^{-1} of BAP in species of the genus *Passiflora*. The concentrations of 0.5 and 2.5 mg L^{-1} of BAP differed significantly in the presence of auxin, however the other doses showed no difference. The lowest mean was observed in the presence of auxin with the highest cytokinin dose.

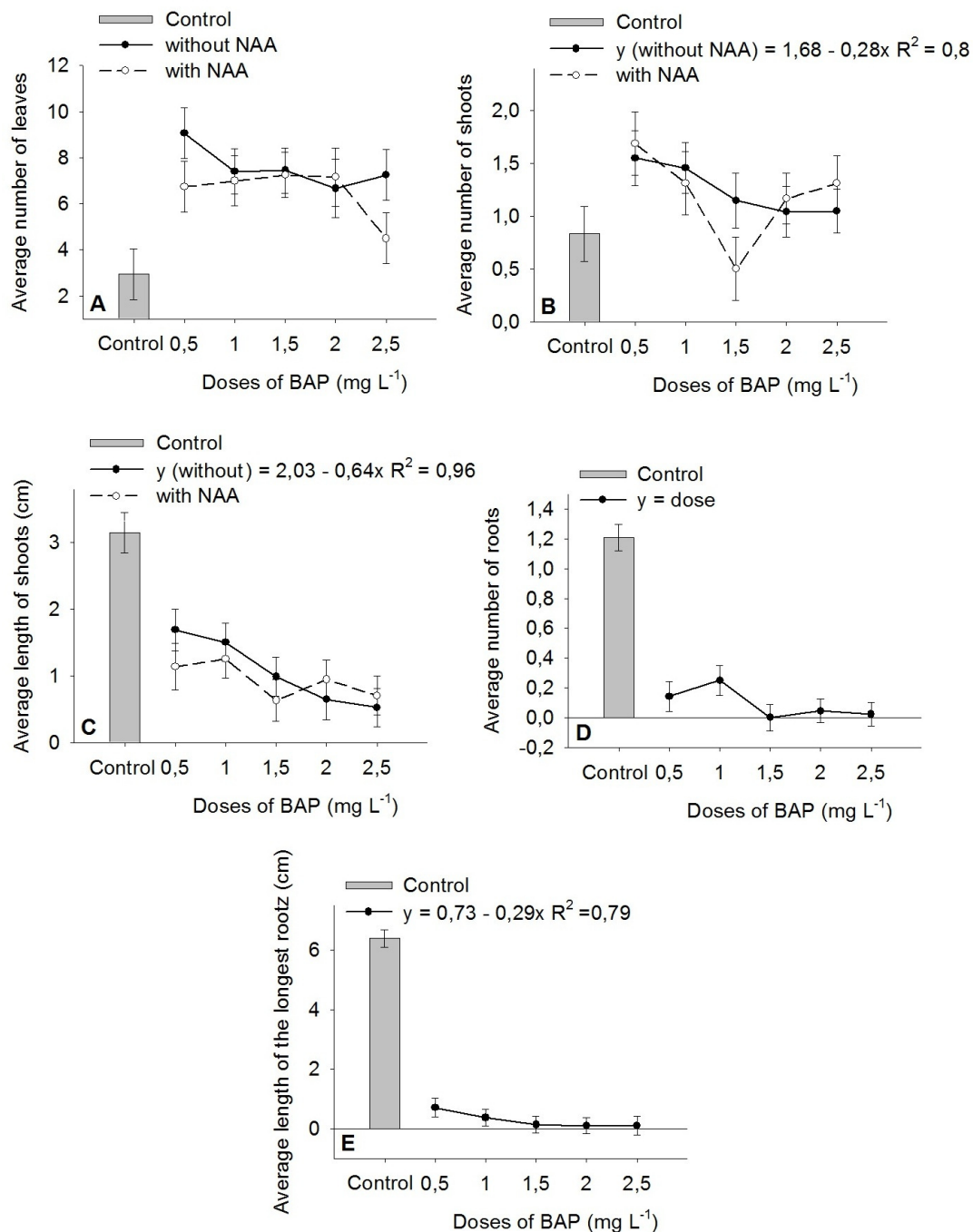


Fig. 1. Average number of leaves (A), average number of shoots (B), average length of shoots (C), average number of roots (D) and average length of the largest root (E), of stem segments with a shoot of yellow passion fruit evaluated at 60 days. Pelotas-RS, 2015.

Vertical bars represent the t-test statistical significant difference ($p \leq 0.05$) or the 95% confidence intervals.

Regarding the number of shoots (Fig. 1B), the highest mean dose was 0.5 mg L⁻¹ of BAP in combination with NAA, and did not differ significantly from the same dose in auxin absence. When comparing the doses, it was observed that explants inoculated in BAP containing media did not differ among the other concentrations. It was verified that comparing the presence or absence of NAA in the culture medium,

only the 1.5 mg L⁻¹ concentration showed a significant difference, which was not observed on the other doses.

Soares [16], using nodal segments from native species plantlets, observed that the species *P. setacea* presented the highest shoot number average at 2.0 mg L⁻¹ of BAP concentration, followed by *P. cincinnata* at the concentration of 1.0 mg L⁻¹ and, again *P. setacea* in medium containing 0.5 mg L⁻¹, finding no significant difference in relation to BAP concentrations. Pacheco and co-authors [17] obtained higher shoot number averages using *P. alata* in MSM medium at BAP concentrations of 2 and 4 mg L⁻¹.

The highest length of shoots was observed when growth regulators were not used, differing statistically from the tested doses (Fig. 1C). Comparing the doses, the highest value was observed at BAP concentration of 0.5 mg L⁻¹ in the absence of NAA, but did not differ from the same dose in the presence of auxin. It was verified that without NAA, the lowest dose did not present a dose difference from 1.0 mg L⁻¹, however, it differed from the others. In the presence of auxin, no difference was observed between doses, differing only from the control where highest value was obtained.

Comparing doses without NAA, a decrease was observed according to the concentrations increase, noticed 74.85% with 2.5 mg L⁻¹ of BAP compared to the lower dose. Similar results were found by Soares [16], who obtained a higher mean in the regulator absence, and also observed a decreasing linear behavior, in which higher concentrations of BAP determined smaller shoot lengths. Anand and co-authors [18] researching with *P. foetida*, observed that the highest sprout length was obtained in MS medium supplemented with 1.5 mg L⁻¹ BAP combined with 0.5 mg L⁻¹ NAA.

Regarding the variables average number of roots (Fig. 1D) and average length of the largest root (Fig. 1E), it was verified statistical significance only for BAP application. The control presented a higher mean for roots number and roots length, differing from the tested concentrations. When comparing doses, 0.5 and 1.0 mg L⁻¹ of BAP did not differ among themselves, but the dose of 1.0 mg L⁻¹ presented difference in relation to the others and characterized the highest average number of roots between doses.

Concerning the mean length of the largest root, the doses did not differ from each other, but a decrease in BAP doses 1.5 and 2.5 mg L⁻¹ of 49.57 and 99.14%, respectively, was observed compared to the lowest concentration. Anand and co-authors [18] studying *P. foetida* observed a greater roots induction in nodal segments when grown in MS medium supplemented with 0.5 mg L⁻¹ of NAA combined with 0.5 mg L⁻¹ of IBA.

4. CONCLUSION

Growth regulators absence promoted a longer shoot length, the highest root length and also the highest number of roots of *P. edulis* f. *flavicarpa* on in vitro multiplication. BAP concentration of 0.5 mg L⁻¹ without NAA addition MS culture medium presented higher number of leaves. The BAP concentration of 0.5 mg L⁻¹ combined with NAA promoted the highest number of shoots. This study developed optimum medium for yellow passion fruit. Different media were observed to be appropriate for the analyzed variables. The procedure can be of great usefulness for mass multiplication and improvement of this economically important plant species.

REFERENCES

1. Lima A de A, Cardoso CEL, Souza J da S, Pires M de M. Marketing of yellow passion fruit. Brasília: EMBRAPA-CNPMPF, 2013. (Boletim, 29). Available: http://www.cnpmf.embrapa.br/publicacoes/produto_em_foco/maracuja_29.pdf. (Accessed on 20 Oct 2014). Portuguese.
2. Carvalho C de, Kist BB, Santos CE dos, Treichel M, Filter CF, Beling RR, editors. Brazilian Fruit Science Yearbook. Santa Cruz do Sul (RS): Editora Gazeta Santa Cruz; 2017. Portuguese.

- 151 3. Bernacci LC, Soares-Scott MD, Junqueira NTV, Passos IRS, Meletti LMM. *Passiflora edulis* Sims:
152 the correct taxonomic way to cite the yellow passion fruit (and of others colors). *Revista Brasileira*
153 *de Fruticultura*. 2008;2(30):566-576.
- 154 4. Meletti LMM, Oliveira JC de, Ruggiero C. Yellow Passion Fruit. Native Fruits Series. Jaboticabal:
155 Funep; 2010. Portuguese.
- 156 5. Ozarowski M, Thiema B. Progress in micropropagation of *Passiflora* spp. to produce medicinal
157 plants: a mini-review. *Revista Brasileira de Farmacognosia*. 2013;(23):937-947.
- 158 6. Moraes TP, Luz JMQ, Silva SM, Resende RF, Silva AS. Applications of tissue culture in medicinal
159 plants. *Revista Brasileira de Plantas Mediciniais*. 2012;1(14):110-121. Portuguese.
- 160 7. Almeida LV da S, Oliveira VJ dos S de, Jacobi CCB de, Almeida WAB de. Medicinal plants and
161 micropropagation as a tool for its expansion and use. *Revista Textura*. 2016;16(9):01-14.
162 Portuguese.
- 163 8. George EF, Hall MA, Klerk G de. Plant propagation by tissue culture. 3rd ed. The Background:
164 Springer; 2008.
- 165 9. Cordeiro IMCC, Lameira AO, Ohashi ST, Rosal LF. Effect of BAP on in vitro shoots proliferation of
166 *Schizolobium amazonicum* Huber ex Ducke (Paricá). *Cerne*. 2004;1(10):118-124. Portuguese.
- 167 10. Saini S, Sharma I, Kaur N, Pati PK. Auxin: a master regulator in plant root development. *Plant Cell*
168 *Reports*. 2013;6(32):741-757.
- 169 11. Bielach A, Duclercq J, Marhavý P, Benková E. Genetic approach towards the identification of
170 auxin-cytokinin crosstalk components involved in root development. *Philosophical Transactions of*
171 *the Royal Society B*. 2012;1595(367):1469-1478.
- 172 12. Muller D, Leyser O. Auxin, cytokinin and the control of shoot branching. *Annals of Botany*.
173 2011;7(107):1203-1212.
- 174 13. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue
175 cultures. *Physiology Plantarum*. 1962;(15):473-497.
- 176 14. Rousseeuw PJ, Leroy AM. Robust Regression and Outlier Detection. 1st ed. New York: John Wiley
177 & Sons; 1987.
- 178 15. Barnett V, Lewis T. Outliers in Statistical Data. 3rd ed. New York: John Wiley & Sons; 1994.
- 179 16. Soares ALC. Multiplication, rooting and in vitro conservation of native passion fruit. Thesis (Master).
180 Minas Gerais: Lavras; 2013. Portuguese
- 181 17. Pacheco G, Garcia R, Lugato D, Vianna M, Mansur E. Plant regeneration callus induction and
182 establishment of cell suspension cultures of *Passiflora alata* Curtis. *Scientia Horticulturae*.
183 2012;1(144):42-47.
- 184 18. Anand SP, Jayakumar E, Jeyachandran R, Nandagobalan V, Doss A. Direct organogenesis of
185 *Passiflora foetida* L. through nodal explants. *Plant Tissue Culture Biotechnology*. 2012;1(22):87-91.
186