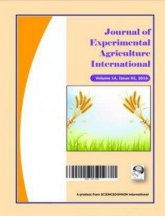

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Cytokinin and Auxin on *In vitro* Multiplication of Yellow Passion Fruit

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Authors' contributions

This work was carried out in collaboration between all authors. Author SIC conducted laboratory work and wrote the manuscript. All other authors contributed equally in data collection, managed the study analyses and discussion. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The objective of this research was to evaluate the effect of growth regulators on *in vitro* multiplication of *Passiflora edulis* f. *flavicarpa*.
Study Design: The 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 the Fruit Plant Propagation Laboratory, Federal University of Pelotas, RS, Brazil. After 60 days of cultivation, the number of leaves, shoots, average shoot length (cm), average number of roots and average length of roots (cm) were evaluated.
Methodology: The Stem segments with a shoot were inoculated on MS medium adding or not 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.

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Results: The length of shoot and longest root, and root number were lower in the presence of BAP and NAA than the control (without BAP and NAA). BAP at 0.5 mg L^{-1} without NAA showed the highest number of leaves and the same dose of BAP with NAA, irrespective of the combination of NAA, showed the highest number of shoots of *P. edulis* f. *flavicarpa* on *in vitro* multiplication.
Conclusion: Cytokinin at low concentration is suitable for the proliferation of shoots and development of leaves. However, cytokinin and auxin are not preferred to the differentiation and growth of the roots. Yellow passion fruit might be efficiently multiplied by using different media for propagation of shoots and differentiation of roots, respectively.

Keywords: Passiflora edulis f. flavicarpa; micropropation; 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 in the world [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. Yet, 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 of cultivation [3,4].

In vitro propagation of *Passiflora* genus has been shown to be viable for commercial 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 can afford alternatives for greater biomass production and ensures the economic interest species perpetuation [6].

The addition of growth regulators, especially auxins and cytokinins, play a very important role in the plant growth mechanism. The combination of auxin and cytokinin and the 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* tissue culture, they promote the overcoming of apical dominance by breaking lateral buds dormancy and thus are related to shoot induction [8].

BAP is as commercially available as 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 tissue culture 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 et al. [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 of 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 research was to evaluate the effect of growth regulators (BAP and NAA) on *in vitro* multiplication of *P. edulis* f. *flavicarpa*.

2. MATERIALS AND METHODS

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

Explants used in this experiment came from yellow passion fruit (*P. edulis* f. *flavicarpa*) already established *in vitro*, and 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 addition of growth regulators.

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 tissue culture [13], supplemented with 100 mg L^{-1} myo-inositol, 30 g L^{-1} sucrose, with or without plant growth regulators, according to the treatments. The pH of the culture medium 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. The autoclaved culture medium of 30 mL was dispensed in sterilized glass flask of 300 mL under aseptic condition.

After the inoculation of explants, the flask was sealed with aluminum foil and Parafilm®, and 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 culture, the number of leaves, shoots and roots, and the length of shoot and the longest root were measured.

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, the average number of roots and average length of the longest root $\sqrt{(x + 0.5)}$ transformation was necessary. The data were then submitted to analysis of variance by F test ($p \leq 0.05$). Observing a statistical significance, the effects of the BAP (with and without NAA) were compared by Tukey test ($p \leq 0.05$), and the MSD (Minimal Significant Difference) 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:

$$y = a + bx;$$

where: y = variable response; a = maximum estimated value for the variable response; b = slope of the line or curve; x = BAP doses (mg L^{-1}). 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

There was a greater increase in the number of leaves in the concentration of 0.5 mg L^{-1} of BAP without NAA (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], who has achieved the highest average per explant using 1.0 mg L^{-1} of BAP in species of the genus *Passiflora*. The number of leaves differed significantly between 0.5 and 2.5 mg L^{-1} in the presence of NAA, although no differences were found between 0.5 mg L^{-1} and the other doses. The lowest mean was observed in the presence of auxin with the highest cytokinin dose.

Regarding the number of shoots (Fig. 1B), the highest mean was found in 0.5 mg L^{-1} of BAP in combination with NAA, and it did not differ significantly from the same dose of BAP without NAA. Comparing the concentrations, 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} BAP showed a significant difference with the other doses.

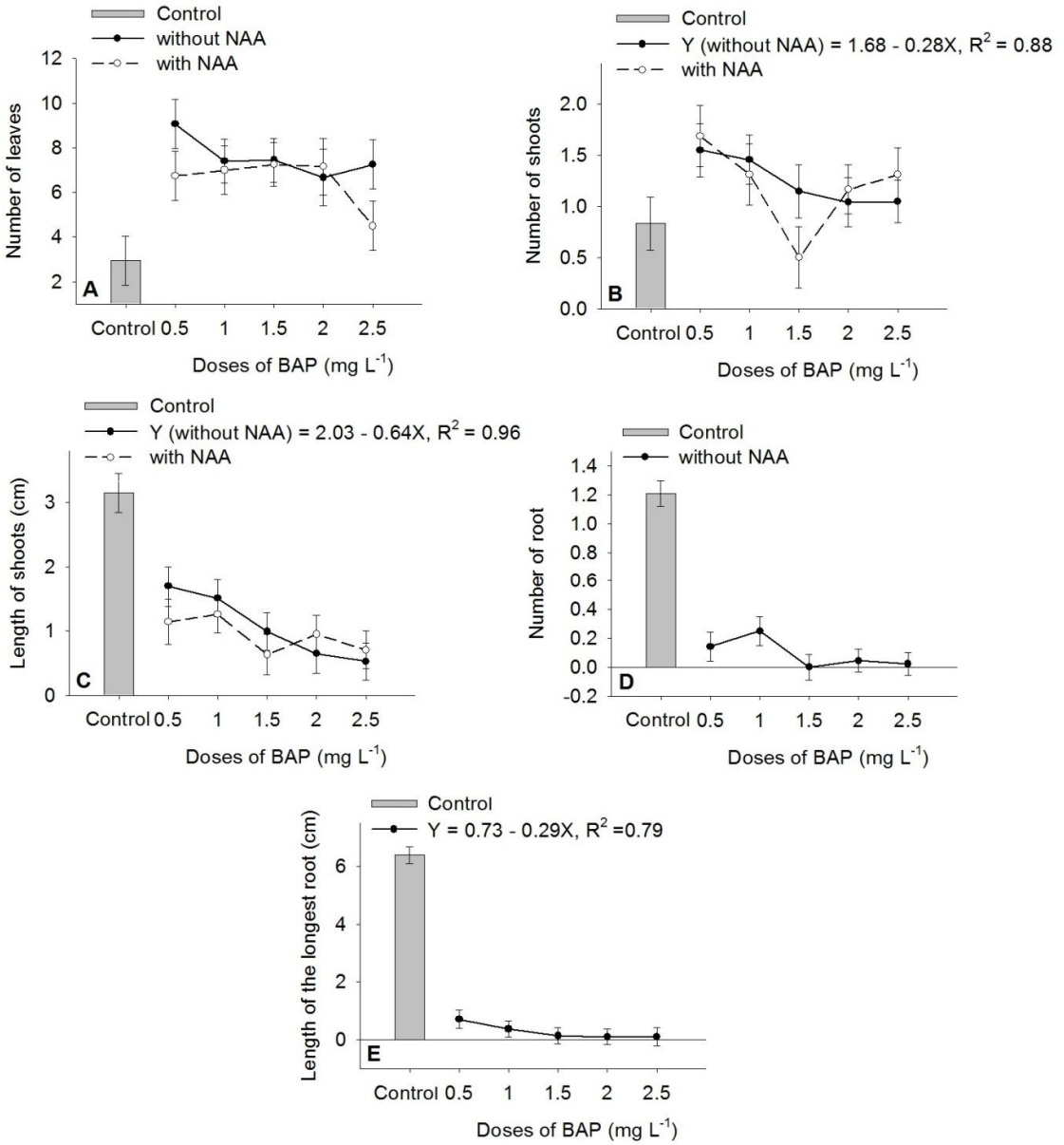

Fig. 1. Average number of leaves (A), number of shoots (B), length of shoots (C), number of roots (D) and length of the longest root (E), of stem segments with a shoot of yellow passion fruit evaluated at 60 days of beginning of culture. Pelotas-RS, 2015

Vertical bars represent the t-test statistical significant difference (p≤0.05) or the 95% confidence intervals

Soares [16], using nodal segments from native *Passiflora* species, reported that the highest shoot number was found at 2.0 mg L^{-1} BAP in *P. setacea*, followed by *P. cincinnata* at the concentration of 1.0 mg L^{-1} and, *P. setacea* in medium containing 0.5 mg L^{-1}, finding no significant difference in relation to BAP concentrations. Pacheco et al. [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 (Fig. 1C). Comparing the doses, the highest value was observed at 0.5 mg L^{-1} BAP in the abscence of NAA, but it did not differ from the same dose in the presence of auxin. It was verified that without

NAA, the effect of 0.5 mg L^{-1} BAP was significantly higher than that of the other doses except 1.0 mg L^{-1}. In the presence of NAA, no difference was observed between BAP doses, differing only from the control where highest value was found.

Regarding the effect of BAP doses, the higher the dose in the culture medium, the shorter the length of the shoots; the length in 2.5 mg L^{-1} was 74.9% of that in 0.5 mg L^{-1}. Similar results were reported by Soares [16], who found a higher mean in the growth regulator absence, and also observed a decreasing linear behavior, in which higher concentrations of BAP resulted smaller shoot lengths. Anand et al. [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 longest root (Fig. 1E), it was verified statistical significance only for BAP application. The highest means of roots number and roots length were found in the control, differing from the tested concentrations. Although there was no significant difference between 0.5 and 1.0 mg L^{-1}, the roots number in 1.0 mg L^{-1} was significantly higher than the other higher doses.

There were no significant differences in the longest root length between the BAP dose, a decrease in BAP doses 1.5 and 2.5 mg L^{-1} of 49.6 and 99.1%, respectively, was observed compared to the lowest concentration. In *P. foetida*, Anand et al. [18] 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

Cytokinin at low concentration is suitable for the proliferation of shoots and development of leaves. However, cytokinin and auxin are not preferred to the differentiation and growth of roots. Yellow passion fruit might be efficiently multiplied by using different media for propagation of shoots and differentiation of roots, respectively.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lima A de A, Cardoso CEL, Souza J da S, Pires M de M. Marketing of yellow passion fruit. Brasília: EMBRAPA-CNPMF; 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.
3. Bernacci LC, Soares-Scott MD, Junqueira NTV, Passos IRS, Meletti LMM. *Passiflora eduli*s Sims: The correct taxonomic way to cite the yellow passion fruit (and of others colors). Revista Brasileira de Fruticultura. 2008;2(30):566-576.
4. Meletti LMM, Oliveira JC de, Ruggiero C. Yellow Passion Fruit. Native Fruits Series. Jaboticabal: Funep; 2010. Portuguese.
5. Ozarowski M, Thiema B. Progress in micropropagation of *Passiflora* spp. to produce medicinal plants: a mini-review. Revista Brasileira de Farmacognosia. 2013;(23):937-947.
6. Morais TP, Luz JMQ, Silva SM, Resende RF, Silva AS. Applications of tissue culture in medicinal plants. Revista Brasileira de Plantas Medicinais. 2012;1(14):110-121. Portuguese.
7. Almeida LV da S, Oliveira VJ dos S de, Jacobi CCB de, Almeida WAB de. Medicinal plants and micropropagation as a tool for its expansion and use. Revista Textura. 2016;16(9):01-14. Portuguese.
8. George EF, Hall MA, Klerk G de. Plant propagation by tissue culture. 3rd ed. The Background: Springer; 2008.
9. Cordeiro IMCC, Lameira AO, Ohashi ST, Rosal LF. Effect of BAP on in vitro shoots proliferation of *Schizolobium amazonicum* Huber ex Ducke (Paricá). Cerne. 2004;1(10):118-124. Portuguese.
10. Saini S, Sharma I, Kaur N, Pati PK. Auxin: a master regulator in plant root development. Plant Cell Reports. 2013; 6(32):741-757.
11. Bielach A, Duclercq J, Marhavý P, Benková E. Genetic approach towards the identification of auxin-cytokinin crosstalk components involved in root development. Philosophical Transactions of the Royal Society B. 2012;1595(367):1469-1478.

12. Muller D, Leyser O. Auxin, cytokinin and the control of shoot branching. Annals of Botany. 2011;7(107):1203-1212.
13. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiology Plantarum. 1962;15:473-497.
14. Rousseeuw PJ, Leroy AM. Robust regression and outlier detection. 1st ed. New York: John Wiley & Sons; 1987.
15. Barnett V, Lewis T. Outliers in Statistical Data. 3rd ed. New York: John Wiley & Sons; 1994.
16. Soares ALC. Multiplication, rooting and in vitro conservation of native passion fruit. Thesis (Master). Minas Gerais: Lavras; 2013. Portuguese
17. Pacheco G, Garcia R, Lugato D, Vianna M, Mansur E. Plant regeneration callus induction and establishment of cell suspension cultures of *Passiflora alata* Curtis. Scientia Horticulturae. 2012; 1(144):42-47.
18. Anand SP, Jayakumar E, Jeyachandran R, Nandagobalan V, Doss A. Direct organogenesis of *Passiflora foetida* L. through nodal explants. Plant Tissue Culture Biotechnology. 2012; 1(22):87-91.

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