Research, Society and Development, v. 11, n. 5, e35511528055, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i5.28055

In vitro rhizogenesis of *Ananas comosus* var. *erectifolius* under influence of synthetic auxins

Rizogênese in vitro de *Ananas comosus* var. *erectifolius* sob influência de auxinas sintéticas

Rizogénesis in vitro de *Ananas comosus* var. *erectifolius* bajo la influencia de auxinas sintéticas

Received: 03/14/2022 | Reviewed: 03/23/2022 | Accept: 04/05/2022 | Published: 04/09/2022

Leonardo Soriano
ORCID: https://orcid.org/0000-0002-8958-4000
University of São Paulo, Brazil
E-mail: lsoriano@usp.br
Carolina Rossi de Oliveira
ORCID: https://orcid.org/0000-0002-3563-4834
University of São Paulo, Brazil
E-mail: carolinna.rossi1987@gmail.com
Fabiana Rezende Muniz
ORCID: https://orcid.org/0000-0001-7002-1032
Suzano S.A., Brazil
E-mail: fabiana.muniz@hotmail.com.br
Isaac Stringueta Machado
ORCID: https://orcid.org/0000-0002-8570-858X
São Paulo State University, Brazil
E-mail: i.machado@unesp.br

Abstract
Ananas comosus var. *erectifolius* is a bromeliad native of the Amazon Complex has aroused great interest of the industries due to a softness, strength and low density of fibers extracted from its leaves. The frequent demand and effective protocols for in vitro regeneration and establishment of species, of commercial and environmental interest, encourages studies of organogenesis induced by growth regulators. The rhizogenesis or in vitro rooting is the last step of the regeneration process and is often considered the greatest challenge to be overcome. Rhizogenesis of *Ananas comosus* var. *erectifolius* was evaluated under the influence of the growth regulators naphthalene acetic acid (NAA) and indole butyric acid (IBA). Seven experiments were performed using different concentrations of growth regulators on solid MS medium under controlled environmental conditions. The results will improve the laboratorial protocols of induction, multiplication, elongation and production of root biomass for *A. comosus* var. *erectifolius*. The in vitro rooting was feasible and better results were observed using 1.0 mg L^{-1} NAA + 1.0 mg L^{-1} IBA.
Keywords: Bromeliaceae; Natural fibers; In vitro rooting; Naphthalene acetic acid; Indole butyric acid.

Resumo
Ananas comosus var. *erectifolius* é uma bromélia nativa do Complexo Amazônico que tem despertado grande interesse das indústrias devido a maciez, resistência e baixa densidade das fibras extraídas de suas folhas. A frequente demanda por protocolos eficazes para o estabelecimento e regeneração in vitro de espécies de interesse comercial e ambiental, estimulam estudos de organogênese induzida por reguladores de crescimento. A rizogênese ou enraizamento in vitro consiste na última etapa do processo de regeneração e, muitas vezes, é considerada o maior desafio a ser superado. A rizogênese de *Ananas comosus* var. *erectifolius* foi avaliada sob influência dos reguladores de crescimento ácido naftaleno acético (ANA) e ácido indolbutírico (AIB). Sete experimentos foram realizados utilizando diferentes concentrações de reguladores de crescimento adicionados em meio MS sólido, sob condições ambientais controladas. Os resultados obtidos irão otimizar os protocolos laboratoriais de indução, multiplicação, alongamento e produção de biomassa radicular para *A. comosus* var. *erectifolius*. O enraizamento in vitro foi viável e os melhores resultados foram observados com 1,0 mg L^{-1} ANA + 1,0 mg L^{-1} AIB.
Palavras-chave: Bromeliaceae; Fibras naturais; Enraizamento in vitro; Ácido naftaleno acético; Ácido indolbutírico.

Resumen
Ananas comosus var. *erectifolius* es una bromelia originaria del Complejo Amazónico que ha despertado gran interés en las industrias debido a la suavidad, resistencia y baja densidad de las fibras extraídas de sus hojas. La demanda frecuente y protocolos efectivos para la regeneración in vitro y establecimiento de especies, de interés comercial y ambiental, incentiva los estudios de organogénesis inducida por reguladores de crecimiento. La rizogénesis o enraizamiento in vitro es el último paso del proceso de regeneración y suele considerarse el mayor reto a superar. Rizogénesis de *Ananas comosus* var. *erectifolius* se evaluó bajo la influencia de los reguladores de crecimiento ácido

naftaleno acético (ANA) y ácido indol butírico (AIB). Se realizaron siete experimentos usando diferentes concentraciones de reguladores de crecimiento en medio MS sólido bajo condiciones ambientales controladas. Los resultados mejorarán los protocolos de laboratorio de inducción, multiplicación, elongación y producción de biomasa de raíces para *A. comosus* var. *erectifolius*. El enraizamiento in vitro fue factible y se observaron mejores resultados utilizando 1,0 mg L^{-1} ANA + 1,0 mg L^{-1} AIB.
Palabras clave: Bromeliaceae; Fibras naturales; Enraizamiento in vitro; Ácido naftaleno acético; Ácido indol butírico.

1. Introduction

Ananas comosus (L.) Merr. var. *erectifolius* (L.B.Sm.) Coppens & F. Leal is a bromeliad native of the Amazon Complex (Leão *et al*, 2009; Gato *et al*, 2019). These plants are herbaceous, monocotyledon of fasciculated root system, easy adaptation to different types of soil and climate change (Oliveira *et al*, 2010). Four different varieties of species can occur being the variety with reddish-purple leaves the best adapted to the edaphic conditions of southeastern Brazil (Leão *et al*, 2009; Garcia *et al*, 2010). As an important genetic resource, especially to be closely related plant species of pineapple, the cp genome sequence of *A. comosus* var. *erectifolius* was recently characterized for future phylogenetic identification and analysis of agronomic traits (Liu; Zhang; He, 2022). Due to the softness, strength and low density of fibers extracted from its leaves, *A. comosus* var. *erectifolius* has aroused great interest of the industries, especially automotive, production of polymeric composites and construction (Mothé; Araújo, 2004; Sena Neto *et al*, 2015; Asim *et al*, 2015; Sena Neto *et al*, 2017; Souza *et al*, 2017).

The increasing replacement of synthetic material by natural fibers has ecological, social, mechanical and economic advantages (Leão; Caraschi; Tan, 2000; Sena Neto *et al*, 2015; Sena Neto *et al*, 2017). In vitro propagation by direct and indirect in vitro organogenesis has shown greater efficiency in producing uniform and healthy plants in large scale. Seedlings produced in vitro are differentiated from native plants in that they have softer leaves and maintain the characteristics of absence of thorns, resulting from the selection work. That makes the harvest of the leaves easier, allowing greater density of plants per hectare, due to greater accessibility at the time of harvest (Gato *et al*, 2019).

Adventitious root induction is promoted by high auxin and low cytokinin levels (Bollmark; Eliasson, 1986; Kuroha *et al*, 2002; Kuroha *et al*, 2006). The most commonly used auxins in rooting media are the indole acetic acid (IAA), naphthalene acetic acid (NAA) and indole butyric acid (IBA). NAA is a synthetic auxin and, like the natural ones, generally promotes the differentiation and elongation of plant cells and tissues in vitro and ex vitro. IBA is often quoted in in vitro propagation studies mainly due to its stability, greater action spectrum in different species and lower toxicity in plants. In a large number of species, similar concentrations of NAA and IBA together promoted higher rooting percentage than each one acting separately (Hinojosa, 2000; Kuroha *et al*, 2006).

The aim of this work was to obtain an efficient laboratory protocol for in vitro rooting of *A. comosus* var. *erectifolius*, essential step for the in vitro propagation process. This process can provide uniform seedlings with desirable agronomic characteristics and with great ecological interest due to the replacement of synthetic fibers.

2. Methodology

The experiment was conducted at Department of Forest Science, Soil and Environment - Faculty of Agronomic Sciences - São Paulo State University, Botucatu, SP, Brazil. Plants of *A. comosus* var. *erectifolius*, purple variety, grown in experimental farms located in the municipalities of Botucatu - SP and São Manuel - SP were used as sources of explants.

Shoots from axillary buds were selected and established in MS medium (Murashige; Skoog, 1962) supplemented with different concentrations of the growth regulators NAA and IBA, in 7 treatments (Table 1).

Table 1. Treatments using the plant growth regulators NAA and IBA to induce in vitro rooting of *A. comosus* var. *erectifolius.*

	Treatments (mg L^{-1})						
	T1	T2	T3	T4	T5	T6	T7
NAA	0	0	1	2	1	1	2
IBA	0	1	1	1	0	2	2

Source: Authors.

The experimental design was completely randomized with 8 replicates per treatment. The methodology used for treatment and disinfection of explants, in vitro establishment of axillary buds and shoot induction was first described by Leão *et al* (2009) and Machado *et al* (2009).

After 45 days under a 16h photoperiod, with light intensity of 1,000 lux and temperature of 27 ºC in a growth chamber (BOD), the plants were collected and the following anatomical and physiological parameters were measure: root length (cm), number of roots and production of fresh (g/root) and dry matter (g/root). The results were compared by statistical analysis using Tukey test (5% probability) (MINITAB Release 14.12.0, Minitab Inc.).

3. Results and Discussion

The overall mean for root length was 3.98 cm. Best results for this parameter were shown by treatments T2 (CR = 4.41 cm), T3 (CR = 4.61 cm) and T4 (CR = 4.45 cm). The average number of roots formed was approximately 8 per explant and the best treatments were T2 (NR = 12), T3 (NR = 10), T4 (NR = 9) and T5 (NR = 9) (Table 2; Figure 1). IBA at the concentration of 1 mg L^{-1} was responsible for the largest induction and root growth in *A. comosus* var. *erectifolius*, regardless of the dose of NAA in the culture medium.

Table 2. Mean values for root length (RL), number of roots (NR), fresh matter (FM) and dry matter (DM) of roots of *A. comosus* var. *erectifolius* after 45 days of in vitro culture, under 7 different treatments (T1-T7)

Treatments	RL	NR	FM	DM
T1	3.45 b	8 ab	0.2240 b	0.0179 b
T2	4.41 a	12 a	0.2584 b	0.0198 ab
T3	4.61 a	10 a	0.4401 a	0.0360 a
T4	4.45 a	9 a	0.2549 b	0.0200 ab
T5	3.76 ab	9 a	0.3784 a	0.0301 a
T6	3.75 ab	6 ab	0.1923 b	0.0162 b
T7	3.40 b	5 b	0.1645 b	0.0123 b

Means followed by the same letter (in the column) did not differ significantly by Tukey test ($p < 0.05\%$). Source: Authors.

Figure 1. Root development of *A. comosus* var. *erectifolius* after 45 days of in vitro culture, under 7 different treatments (T1-T7).

Source: Authors.

Martins *et al* (2013) studied the isolated effect of IBA on rooting of *Neoregelia concentrica* (Vellozo) L. B. Smith and observed an average length of roots of 2.27 cm with the addition of 2 µM L^{-1} in MS medium supplemented with 10 mg L^{-1} of citric acid. However, some authors have reported isolated action of NAA on root formation of in vitro shoots through direct organogenesis of *A. comosus* var. *erectifolius* (Leão *et al*, 2009) and also of related species such as pineapple (Macedo *et al*, 2003; Moraes *et al*, 2010; Pineda *et al*, 2012; Sani et al, 2019; Fernando *et al*, 2020; Kornatskiy, 2020; Cacaï *et al*, 2021; Dhurve *et al*, 2021) and ornamental bromeliads (Pierik; Steegmans; Hendriks, 1984; Mendes *et al*, 2007; Martins *et al*, 2013; Martins *et al*, 2020).

Macedo *et al* (2003) confirmed the stimulation of NAA (0.24 mg L^{-1}) on root formation of pineapple L. Merrill, however, root length was higher in the absence of the growth regulator. Nevertheless, Martins *et al* (2020) reported the essentiality of NAA auxin in the formation, growth and fresh mass production of *Alcantarea imperialis* roots. The same result was observed by Moraes *et al* (2010), who found an increase of the number and decrease of the length of the roots of pineapple plantlets cv. EMEPA 1, through supplementation of MS medium with 1 mg L^{-1} NAA. Cacaï *et al* (2021) observed a significant increase of root formation in plants regenerated from somatic embryos of pineapple using 0.5 mg L^{-1} ANA + 1.0 mg L^{-1} BAP (benzylaminopurine) supplemented on MS basal medium.

The production of fresh and dry matter also showed significant differences among treatments, with greater biomass production in T3 (FM = 0.4401 g and DM = 0.0360 g) and T5 (FM = 0.3784 g and DM = 0.0301 g) (Table 2). NAA assumed an important role for these parameters. Treatments with 1 mg L^{-1} NAA were significantly higher. Treatment 5 (T5), without IBA, suggests the isolated effect of NAA in biomass accumulation.

Martins *et al* (2013), when comparing the effect of NAA in relation to IBA on rooting of the bromeliad *N. concentrica* after 30 days, observed a higher number of roots in shoots cultured in concentrations higher than 1 µM of NAA in relation to AIB. The control treatment (T1) showed the possibility of rooting without the addition of exogenous growth regulators. Although Sani *et al* (2019) observed root morphogenesis on in vitro plants of *A. comosus* (L.) var. Smooth Cayenne through the combination of BAP with activated charcoal, treatments without growth regulator supplementation produced a high number of vigorous roots. This is probably due to the physiological activity of IAA within the cultured tissue. Induction of in vitro morphogenesis and growth of roots depend not only on auxin added to the medium but also on the interaction of the

endogenous concentrations of IAA (Pasqual *et al*, 2001). Treatment 7 (T7) with 2 mg L^{-1} NAA + 2 mg L^{-1} IBA showed the lowest values in all parameters evaluated. Grattapaglia and Machado (1998) state that root growth can be inhibited by an excess of auxin in the culture medium and high levels of endogenous NAA may impair the stretching step and, hence, the accumulation of biomass. Adeoye *et al* (2020) observed highest average mean number for root emergence (5.3 roots per shoot) of *A. comosus* (L.) var. Smooth Cayenne in combination with 2 mg L^{-1} IBA and 2 mg L^{-1} NAA on MS basal medium supplemented with vitamins.

For our results, in general, the treatment 3 (1 mg L^{-1} NAA + 1 mg L^{-1} IBA) showed greater induction of initiation, elongation and production of root biomass.

4. Final Considerations

The results of this study demonstrate the in vitro rooting process of *A. comosus* var. *erectifolius* was feasible under the conditions evaluated; solid MS culture medium, modified by the presence of the growth regulators, enabled the development at all stages of root formation; and a hormonal balance 1/1 (1 mg L^{-1} NAA + 1 mg L^{-1} IBA) showed higher efficiency in inducing and developing roots (multiplication, elongation and biomass production).

Certainly, this rhizogenesis protocol will be useful for researches that requires a complete regeneration of in vitro plants, such as micropropagation. However, new investigations are necessary, especially through molecular techniques, to elucidate the action of growth regulators and their synergistic process in the morphogenesis of *A. comosus*.

Acknowledgments

The authors acknowledge São Paulo Research Foundation (FAPESP) for granting fellowship to LS and to the company Pematec Triangel do Brazil for providing the seedlings.

References

Adeoye, B. A., Lawyer, E. F., Hassan, K. O., Ilesanmi, A. O., Richard-Olebe, T. C., Oyedeji, T. T., Aderemi, T. A., Ajongbolo, F. B., & Adedeji, A. A. (2020). Optimization of Plant Growth Regulator (PGR) on *in vitro* propagation of pineapple (*Ananas comosus* (L.) var. Smooth Cayenne. *International Journal of Recent Research in Life Sciences*, 7(1), 13-20.

Asim, M., Abdan, K., Jawaid, M., Nasir, M., Dashtizadeh, Z., Ishak, M. R., & Hoque, M. E. (2015). A review on pineapple leaves fiber and its composites. *International Journal of Polymer Science*, 2015(1), 1-16. doi.org/10.1155/2015/950567

Bollmark, M. & Eliasson, L. (1986). Effects of exogenous cytokinins on root formation in pea cuttings. *Physiology Plants*, 68(1), 662–666.

Cacaï, G. H. T., Ahokpossi, B. A. M., Houédjissin, S. S., Houngue, J. A., Badou, B. T., Ahanhanzo, C. (2021). Plant regeneration through somatic embryogenesis in two cultigroups of pineapple (*Ananas comosus* L.). *Research Square*, 1-18. DOI: 10.21203/rs.3.rs-244247/v1

Dhurve, L., Kumar, K. A., Bhaskar, J., Sobhana, A., Francies, R. M., & Mathew, D. (2021). Wide variability among the 'Mauritius' somaclones demonstrates somaclonal variation as a promising improvement strategy in pineapple (*Ananas comosus* L.). *Plant Cell Tissue Organ Culture*, 145(1), 701-705. doi.org/10.1007/s11240-021-02022-5

Fernando, Y., Harahap, F., Diky S., & Rosmayati, D. (2020). In Vitro Propagation of Pineapple (*Ananas comosus* L.) Shoots from Sipahutar North Sumatera Indonesia. The International Conference on Sciences and Technology Applications *Journal of Physics*, 1485, 1-9. doi:10.1088/1742-6596/1485/1/012042

Gato, A. M. G., Silva, D., Ferreira, F. F., Pinheiro, E. N., Rodrigues, D. C., Assunção, L. M., Silva, E. L., & Marinho, V. R. P. (2019). Efeitos de diferentes reguladores de crescimento na produção de mudas micropropagadas de *Ananas erectifolius* L.B. Sm. *Scientia Amazonia*, 8(3), 1-7.

Garcia, C. W., Machado, I. S., Almeida, B. O., & Arcalá, L. F. L. (2010). Balanços de fontes nitrogenadas amoniacais e nítricas na micropropagação de curauá. *Revista Brasileira de Oleaginosas e Fibrosas*, 14(1), 125-133.

Grattapaglia, D. & Machado, M. A. Micropropagação. In: Torres, A. C., Caldas, L. S., Buso, J. A. (1998). Cultura de tecidos e transformação genética de plantas. Brasília: Embrapa.

Hinojosa, G. F. Auxinas. In: Barrueto Cid, L. P. (2000). Introdução dos hormônios vegetais. Brasília: Embrapa Recursos Genéticos e Biotecnologia.

Kornatskiy, S. A. (2020). Pineapple micropropagation (*Ananas comosus* L. Smooth Cayenne) and plant growth features in the process of adaptation in hydroponics. *Journal of Critical Reviews*, 7(8), 3228-3234.

Kuroha, T., Kato, H., Asami, T., Yoshida, S., Kamada, H., & Satoh, S. (2002). A trans-zeatin riboside in root xylem sap negatively regulates adventitious root formation on cucumber hypocotyls. *Journal Experimental Botany*, 53(1), 2193–2200.

Kuroha, T., Ueguchi, C., Sakakibara, H., & Satoh, S. (2006). Cytokinin Receptors are required for normal development of auxin-transporting vascular tissues in the hypocotyl but not in adventitious roots. *Plant and Cell Physiology*, 47(2), 234-243.

Leão, A. L., Caraschi, J. C., & Tan, I. H. Curaua fiber - a tropical natural fibers from Amazon potential and applications in composites. In: Mattoso, L.H.C., Leão, A.L., & Frollini, E. (2000). Natural Polymers and Agrofibers Bases Composites. São Carlos: Embrapa Instrumentação Agropecuária.

Leão, A. L., Machado, I. S., Souza, S., & Soriano, L. (2009). Production of curaua (*Ananas erectifolius* LB Smith) fibers for industrial applications: characterization and micropropagation. *Acta Horticulturae*, 822(1), 227-238.

Liu, C., Zhang, W., & He, Y. (2022). The complete chloroplast genome of *Ananas comosus* var. *erectifolius* (L.B. Smith) Coppens & Leal. Resources. *Mitochondrial DNA B Resources*, 7(3), 431-433.

Macedo, C. E. C., Silva, M. G., Nobrega, F. S., Martins, P. M., Barroso, P. A. V., & Alloufa, M. A. I. (2003). Concentrações de ANA e BAP na micropropagação de abacaxizeiro L. Merrill (*Ananas comosus*) e no cultivo hidropônico das plântulas obtidas *in vitro*. *Revista Brasileira de Fruticultura*, 25(1), 501-504.

Machado, I. S., Gomes, A. C., Bertozzo, F., Soriano, L., & Arcala, L. F. L. (2009). Induction of callus formation in *in vitro* curaua meristems. *Revista Brasileira de Oleaginosas e Fibrosas*, 13(1), 83-90.

Martins, J. P. R., Rodrigues, L. C. A., Silva, T. S., Gontijo, A. B. P,L., & Falqueto, A. R. (2020). Modulation of the anatomical and physiological responses of in vitro grown *Alcantarea imperialis* induced by NAA and residual effects of BAP. *Ornamental Horticulture*, 26(2), 283-297.

Martins, J. P. R., Schimildt, E. R., Alexandre, R. S., Santos, B. R., & Magevski, G. C. (2013). Effect of synthetic auxins on *in vitro* and *ex vitro* bromeliad rooting. *Pesquisa Agropecuária Tropical*, 43(2),138-146.

Mendes, G. C., Soares, C. Q. G., Braga, V. F., Pinto, L. C., Santana, R., Viccini, L. F., & Peixoto, P. H. P. (2007). Enraizamento *in vitro* de *Vriesea cacuminis* L. B. Smith (*Bromeliaceae*) do Parque Estadual do Ibitipoca, Minas Gerais, Brasil. *Revista Brasileira de Biociências*, 5(2), 969-971.

Moraes, A. M., Almeida, F. A. C., Bruno, R. L. A., Cazé Filho, J., Nunes, S.T., & Gomes, J.P. (2010). Micropropagação de abacaxizeiro cv. *Emepa 1*. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 14(9), 932-936.

Mothé, C. G. & Araújo, C. R. (2004). Caracterização térmica e mecânica de compósitos de poliuretano com fibras de curauá. *Polímeros: Ciência e Tecnologia*, 14(4), 274-278.

Murashige, T. S. & Skoog, F. A. (1962). Revised medium for rapid growth bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 15(1), 473-497.

Pasqual, M. (2001). Cultura de tecidos. Lavras: UFLA/FAEPE.

Pierik, R. L. M., Steegmans, H. H. M., & Hendriks, J. (1984). The influence of naphthaleneacetic acid on the growth of in vitro cultivated seedling of *Bromeliaceae. Scientia Horticulturae*, 24(1), 193-199.

Pineda, A., Vargas, T. E., Escala, M., & García, E. (2012). Organogenesis *in vitro* in pineapple *'Red Spanish'* and foliar morpho-anatomy of plants obtained in the process. *Bioagro*, 24(3), 175-186.

Oliveira, Y., Anselmini, J. I., Cuquel, F. L., Pinto, F., & Quoirin, M. (2010). Pré-aclimatização *in vitro* de abacaxi ornamental. *Ciência e Agrotecnologia*, 34(1), 1647-1653.

Sani, L. A., Usman, I. S., Nasir, A. U. & Abdulmalik, M. M. (2019). Micropropagation of pineapple (*Ananas comosus* L. var. Smooth Cayenne) in temporary immersion bioreactor system (TIPS). *Bayero Journal of Pure and Applied Sciences*, 12(2), 207-209.

Sena Neto, A. R. S., Claro, P. I. C., Souza, F. V. D., Mattoso, L. H. C., & Marconcini, J. M. (2017). Poly (lactic acid) composites reinforced with leaf fibers from ornamental variety of hybrid pineapple (Potyra). *Polymer Composites*, 38(1), 1228-1235.

Sena Neto, A. R., Araujo, M. A. M., Barboza, R. M. P., Fonseca, A. S., Tonoli, G. H. D., Souza, F. V. D., Mattoso, L. H. C., & Marconcini, J. M. (2015). Comparative study of 12 pineapple leaf fiber varieties for use as mechanical reinforcement in polymer composites. *Industrial Crops and Products*, 64(1), 68-78.

Souza, C. P. F., Ferreira, C. F., Souza, E. H., Sena Neto, A. R., Marconcini, J. M., Ledo, C. A. S., & Souza, F. V. D. (2017). Genetic diversity and ISSR marker association with the quality of pineapple fiber for use in industry. *Industrial Crops and Products*, 104(1), 263-268.