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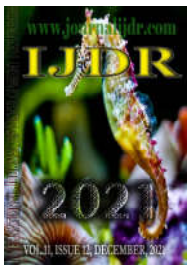

RESEARCH ARTICLE OPEN ACCESS

IN VITRO PROPAGATION OF PIPER HISPIDUM BY SOMATIC EMBRYOGENESIS IN LEAF EXPLANTS

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ABSTRACT

Purpose: A protocol for regeneration of *P. hispidum* through somatic embryogenesis in leaf explants is described. Under aseptic conditions, the leaves were cut into 1.0 cm^2 explants. These explants were inoculated into test tubes with 10.0 mL of an Murashige and Skoog (MS) basal culture medium supplemented with 30.0 g L^{-1} sucrose, 6.0 g L^{-1} agar and a factorial combination of the growth regulators 2,4-Dichlorophenoxyacetic acid (2,4-D) and 6-Benzylaminopurine (BA), both at 0.0, 1.0, 2.0, 3.0 and 4.0 mg L^{-1}, totalizing 25 treatments. The pH was adjusted to 5.8 and the medium autoclaved at 121°C for 20 minutes. After 75 days, the average number of cotyledonary somatic embryos in each explant was recorded.Maximum number of 9.2 cotyledonary embryos per explant was observed with the combination of 2.0 mg L^{-1} BA + 2.0 mg L^{-1} 2,4-D. The subculture of the cotyledonary embryos to a medium without growth regulators resulted in 95% conversion into plantlets, which were acclimatized with 100% survival.

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INTRODUCTION

Piper hispidum Swingle is a shrub native to the lowlands of Mexico, of pan-tropical occurrence. It is distributed throughout the Antilles, and Central and South America, including all Brazilian geographic regions, where is known as "jaborandi" and "falso-jaborandi". The species is used in South American traditional Pharmacopeias for many purposes. In Peru, its leaves are traditionally used by the Chayahuitas, an Amazonian ethnic group, as poultices for healing wounds and to treat the symptoms of cutaneous leishmaniosis (Estevez et al., 2007). In Guatemala, it is known as "puchuq" and used by the Q'eqchi people as a tea to treat dysmenorrhea, amenorrhea, and body aches. In Nicaragua, it is known as "cordoncillo" and also used to ease aches and pains, in Peru to regulate menstruation, and in the Amazon region to treat urinary infections (Michel et al., 2007). In Colombia, a leaf decoction is used to treat malaria (Morton, 1981). Its use as an astringent, diuretic, stimulant, for unblocking the liver and stopping hemorrhages has also been described (Orlandelli et al., 2012). In Brazilian Amazon, *P. hispidum* is known by the vernacular names of "matico" and "aperta-ruão" and its leaf infusion is used in the folk medicine as a diuretic and anti-hemorrhagic (Silva et al., 2014). In terms of chemical constituents, *P. hispidum* is reported to contain amides, benzenes, benzoic acids, flavonoids and volatile oils, which have significant antifungal, antimicrobial, antiplasmodial, leishmanicidal and insecticidal activities (Michel et al., 2010; Silva et al., 2014).

The potential use of *P. hispidum* compounds in medicine and agriculture has encouraged chemical and pharmacological researches. However, collection of *Piper* species from the wild for folk medicine purposes, in addiction to deforestation of tropical forests has diminished their populations and even threatened their very existence, leading to a depletion of the irreplaceable genetic diversity (Ahmad et al., 2011; Rani and Dantu, 2012; Basak et al., 2014). Traditional propagation of *Piper* species has not proved to be efficient, due to poor seed viability, seed recalcitrance, low rates of germination, and scanty or delayed rooting of cuttings, evidencing the need of alternative methods of propagation (Abbasi et al., 2010; Ahmad et al., 2014; Padham, 2015). The objective of the present research was the establishment of a protocol for *in vitro* propagation of *P. hispidum* from leaf explants through somatic embryogenesis.

MATERIAL AND METHODS

The experiments were carried out at the Plant Tissue Culture Laboratory at Embrapa (Brazilian Agricultural Research Corporation) in Porto Velho, Brazil. Leaves of *Piper hispidum* were collected at the experimental field from two years old plants and submitted to disinfestation procedures by washing with running tap water and a detergent agent for five minutes, immersion in 70% ethanol for one minute and in a 1.5% (v/v) sodium hypochlorite solution for 15 minutes, and then rinsed three times with sterile water. Under aseptic conditions, the leaves were cut into 1.0 cm^2 explants. These explants were inoculated into test tubes with 10.0 mL of an Murashige and

Skoog (MS) basal culture medium supplemented with 30.0 g L^{-1} sucrose, 6.0 g L^{-1} agar and a factorial combination of the growth regulators 2,4-Dichlorophenoxyacetic acid (2,4-D) and 6-Benzylaminopurine (BA), both at 0.0, 1.0, 2.0, 3.0 and 4.0 mg L^{-1}, totalizing 25 treatments. The pH was adjusted to 5.8 and the medium autoclaved at 121°C for 20 minutes. After 75 days, the average number of cotyledonary somatic embryos in each explant was recorded.These data were submitted to analyses of variance and Tukey test at 5% of probability. The embryos were transferred to an MS medium lacking growth regulators, at the described conditions, where they were kept for two months to give rise to plantlets. After that the plantlets were submitted to acclimatization in a greenhouse, for more two months, in pots containing Plantmax® substrate, under 50% shading and sprinkler irrigation three times a day for 15 minutes.

RESULTS AND DISCUSSION

Leaf explants appeared swollen in the first 7-14 days of culture, and then many globular embryos were initiated in the margins of the explants. At 30 days of culture, it was possible to recognize heart-shaped embryos, and at 45 days most of the embryos were in the phase torpedo. At 75 days almost all explants had converted into cotyledonary embryos. As can be observed in the Table 1 the use of BA or 2,4-D alone did not induce formation of somatic embryos, evidencing the need of combination of exogenous auxins and cytokinins to promote this physiological event.

Table 1. Average number of cotyledonary somatic embryos in leaf explants of *P. hispidum* cultivated for 75 days in MS medium with factorial combinations of the growth regulators 2,4-D and BA

BA (mg L^{-1})	2,4-D (mg L^{-1})				
	0.0	1.0	2.0	3.0	4.0
0.0	0.00 ^{aA}	0.00 ^{aB}	0.00 ^{aC}	0.00 ^{aC}	0.00 ^{aC}
1.0	0.00 ^{bA}	1.27 ^{bB}	3.65 ^{aB}	1.98 ^{abBC}	1.00 ^{bBC}
2.0	0.00 ^{cA}	4.23 ^{bA}	9.20 ^{aA}	5.77 ^{bA}	3,99 ^{bA}
3.0	0.00 ^{cA}	1.75 ^{abcB}	3.55 ^{aB}	2.15 ^{abB}	2.20 ^{abB}
4.0	0.00 ^{bA}	0.66 ^{abB}	2.01 ^{aBC}	1.03 ^{abBC}	0,00 ^{bC}

*Averages followed by the same capital letter in the columns or small letter in the rows do not differ significantly at 5% probability by Tukey's test.

The same was observed by Souza (2020), who reported efficient differentiation of somatic embryos in *P. hispidinervum* and *P. aduncum* with a combination of 2.5 mg L^{-1} BA + 10.0 mg L^{-1} ANA (naphthaleneacetic acid). However, Yusuf et al. (2001) achieved somatic embryos in *P. colubrinum* by supplementing an MS medium with two cytokinins: 0.5 mg L^{-1} BA + 0.1 mg L^{-1} Kin (kinetin) and inferred that the lack of exogenous auxins was compensated by their endogenous level in the explants. The use of both regulators at higher concentrations gradually inhibited initiation of embryos, with no noticeable changes in the explants submitted to 4.0 mg L^{-1} BA + 4.0 mg L^{-1} 2,4-D. The same was observed by Yusuf et al. (2001), with concentrations of BA above 0.5 mg L^{-1}. The combination of 2,4-D and BA induced embryos in almost all explants, in variable proportions. The concentrations of 2.0 mg L^{-1} BA + 2.0 mg L^{-1} 2,4-D promoted the highest average number of 9.2 cotyledonary embryos per explant. This result can be considered a very high level of propagation, as a single leaf can give origin to more than 20 explants, what means a potential regeneration of 180 plants from one single leaf. The subculture of the cotyledonary embryos to a medium without growth regulators resulted in 95% conversion into plantlets, which were acclimatized with 100% survival of plants.

CONCLUSION

Induction of somatic embryos in *P. hispidum*leaf explants can be achieved in MS medium supplemented with the combination 2.0 mg L^{-1} BA + 2.0 mg L^{-1} 2,4-D.

The somatic embryos develop into plantlets, which can be successfully acclimatized to give rise to plants.

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REFERENCES

Abbasi BH, Ahmad N, Fazal H and Mahmood T. Conventional and modern propagation techniques in *Piper nigrum*. *Journal of Medicinal Plants Research* 4(1): 7-12, 2010.

Ahmad N, Abbasi BH, Fazal H, Khan MA and Afridi MS. Effect of reverse photoperiod on in vitro regeneration and piperine production in *Piper nigrum* L. *Comptes Rendus Biologies*, 337: 19-28, 2014.

Ahmad N, Guo B, Fazal H, Abbasi BH, Liu CZ, Mahmood T and Shinwari ZK. Feasible plant regeneration in black pepper from petiole explants. *Journal of Medicinal Plants Research,* 5(18): 4590-4595, 2011.

Basak UC, Dash D, Jena GJP and Mahapatra AK.New technique for adventitious rooting and clonal propagation of *Piper longum* L. (pippali) through leaf cuttings. *African Journal of Plant Science,* 8(2):108-112, 2014.

Estevez Y, Castillo D, Pisango T, Arevalo J, Rojas R, Alban J, Deharo E, Bourdy G and Sauvain M. Evaluation of the leishmanicidal activity of plants used by Peruvian Chayahuita ethnic group. *Journal of Ethnopharmacology,* 114: 254–259, 2007.

Michel J, Duarte RE, Bolton JL, Huang Y, Caceres A, Veliz M, Soejarto DD andMahady GB. Medical potential of plants used by the Q'eqchi Maya of Livingston, Guatemala for the treatment of women's health complaints. *Journal of Ethnopharmacology,* 114: 92-101, 2007.

Michel JL, Chenb Y, Zhang H, Huang Y, Krunic A, Orjala J, Veliz M, Soni KK, Soejarto DD, Caceres A, Perez A and Mahady GB. Estrogenic and serotonergic butenolides from the leaves of *Piper hispidum*Swingle (Piperaceae). *Journal of Ethnopharmacology,* 129: 220–226, 2010.

Morton JF. Atlas of Medicinal Plants of Middle America, Bahamas to Yucatan.Charles C. Thomas Publisher, Springfield, Illinois, 1981.

Orlandelli RC, Alberto RN, Rubin Filho CJ and Pamphile JA. Diversity of endophytic fungal community associated with *Piper hispidum* (Piperaceae) leaves. *Genetics and Molecular Research*, 11(2): 1575-1585, 2012.

Padham B. Regeneration of plantlets of *Piper longum* L. through in vitro culture from nodal segments. *Journal of Applied Biology and Biotechnology,* 3(05):035-039, 2015.

Rani D and Dantu PK. Direct shoot regeneration from nodal, internodal and petiolar segments of *Piper longum* L. and in vitro conservation of indexed plantlets. Plant Cell, Tissue and Organ Culture, 109: 9-17, 2012.

Silva JKR, Pinto LC, Burbano RMR, Montenegro RC, Guimarães EF, Andrade EHA, Maia JGS. Essential oils of Amazon *Piper* species and their cytotoxic, antifungal, antioxidant and anti-cholinesterase activities.Industrial Crops and Products, 58: 55-60, 2014.

Souza PCA, et al. Somatic embryogenesis and plant regeneration in *Piper aduncum* L. In Vitro Cellular & Developmental Biology – Plant, 56: 618–633, 2020.

Yusuf A, Tyagi RK, Malik SK, 2001. Somatic embryogenesis and plantlet regeneration from leaf segments of *Piper colubrinum*. Plant Cell, Tissue and Organ Culture, 65: 255–258, 2001.

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