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BIOLOGY

Protocol for *in vitro* rooting of *Pyrus comunnis* rootstocks

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ABSTRACT: Effective protocols for *in vitro* rooting for woody fruit trees are still a challenge for *in vitro* seedling production, especially when there is a need to insert new cultivars or rootstocks. These protocols are essential to accelerate studies in plant breeding programs and for seedling distribution. This study evaluated the use of 6-Benzylaminopurine (IBA) in *in vitro* rooting of *Pyrus comunnis* rootstocks, clones 'OHxF87' and Pyrodwarf. Explant exposure times (0, 24, 48, 72, and 96 hours) to 20 mg L^{-1} IBA were tested for *in vitro* rooting. The exposure to IBA resulted in rooting rates above 80%, surpassing some results reported in the literature. The 24-hour treatment provided 81,81% survival, leading to an average growth of five roots with 19 mm length, for 'OHxF87' rootstock. The same exposure time resulted in the highest survival rate (75%) and the highest mean root number, seven roots per plant with 10 mm length, for 'PDW' rootstock. Root formation did not occur in the absence of synthetic auxin. Therefore, it can be concluded that a 24-hour exposure at 20 mg L^{-1} IBA was sufficient to promote *in vitro* rooting in 'OHxF87' and Pyrodwarf rootstocks'.
Key words: 'OHxF87', 'PDW', plant tissue culture, Pyrodwarf, seedling production, 6-Benzylaminopurine.

Protocolo de enraizamento in vitro de porta-enxertos de *Pyrus comunnis*

RESUMO: Protocolos eficazes de enraizamento *in vitro* de frutíferas lenhosas ainda são um desafio para produção de mudas *in vitro*, especialmente quando há necessidade de inserção de novas cultivares ou porta-enxerto. Esses protocolos são essenciais para acelerar estudos nos programas de melhoramento genético e também para distribuição posterior das mudas. Nesse sentido, o objetivo deste estudo foi avaliar a utilização da 6-Benzilaminopurina no enraizamento *in vitro* de porta-enxerto *Pirus comunnis*, clones 'OHxF87' e Pyrodwarf. Para o enraizamento, foi testado o tempo de exposição dos explantes ao AIB. Para tanto, foram utilizados 20 mg L^{-1} do fitohormônio nas horas 0, 24, 48, 72 e 96 horas. A exposição ao AIB resultou em taxas de enraizamento acima de 80%, superando alguns resultados encontrados na literatura. Para o porta-enxerto 'OHxF87', o tratamento de 24 horas proporcionou 81,81% de sobrevivência, promovendo em média cinco raízes com comprimento de 19 mm. O mesmo tratamento para o porta-enxerto 'PDW' resultou na maior taxa de sobrevivência (75%), bem como no maior número médio de raízes, sete raízes por planta, com comprimento de 10 mm. Na ausência de auxina sintética, a formação de raízes não ocorreu. Assim sendo, podemos concluir que o tempo de exposição de 24 horas a 20 mg L^{-1} de IBA foi suficiente para promover o enraizamento de porta-enxertos *Pyrus communis* de 'OHxF87' e 'PDW'.
Palavras-chave: cultura de tecidos vegetais, OHxF87, PDW, Pyrodwarf; Produção de mudas, 6-Benzylaminopurine.

Pear is among the temperate climate fruits best accepted by the domestic consumer market, being one of the main fruits imported by Brazil. One of the limitations in the cultivation of this fruit tree in Brazil is the lack of genetic material (RUFATO et al., 2012). In this sense, new materials have been studied in Brazilian plant breeding programs, for example, rootstocks 'OHxF87' and Pyrodwarf ('PDW), promising in high density plantations.

The series of clones OHxF (Old Home x Farmingdale) originates from *Pyrus communis*, helps in the precocity, yield and quality of some European pear cultivars (ERCISLI et al., 2006). 'OHxF87' clone is one of the best in the series, of semi-dwarf size and compatible with most European and Asian pear varieties (APAL, 2019). Similarly, Pyrodwarf or 'PDW' clone (*Old Home* x *Bonne Luise d'Avranches*) also originates from *P. communis*, has

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good compatibility with European and Asian pear varieties, in addition to low susceptibility to iron chlorosis (WSU, 2019).

Effective protocols for *in vitro* rooting for woody fruit trees are still a challenge for *in vitro* seedling production, especially when there is a need to insert new cultivars or rootstocks. These protocols are essential to accelerate studies in plant breeding programs and for seedling distribution. Concentrations of this plant growth regulator in the forms of indole-3-acetic acid (AIA), indole-3-butyric acid (IBA), naphthalene-acetic acid (NAA), have guaranteed success in rooting cultivars and genotypes of different pear species, such as *P. communis*, *P. pyrifolia, P. calleryana, P. amygdaliformis, P. pyraster, P. syriaca, P. betulifolia,* and *P. bretschneideri* (BELL & REED, 2002).

The results reported in the recent literature demonstrated that, for different Pyrus species, *in vitro* rooting does not occur without the use of synthetic hormonal regulators, and rooting efficiency is dependent on genotype. Therefore, this study evaluated the 6-Benzylaminopurine (IBA) *in vitro* rooting of *P. comunnis* rootstocks, 'OHxF87' and 'PDW' clones.

The plant material was established in QL medium (LEBLAY et al., 1991) at the Laboratory of Plant Micropropagation at Santa Catarina State University. For the multiplication protocol, MS culture medium was used (MURASHIGE & SKOOG, 1962), containing 30 mg L^{-1} of sucrose and 5.5 mg L^{-1} of agar, supplemented with 1.5 mg L^{-1} BAP and 0.1 mg L^{-1} IBA, pH adjusted to 5.8. A total of 20 mL of medium solution was added to the test tubes, which were kept in a growth room at 24 °C with a 16-hour photoperiod (40 - 56 µmol m^{-2} s^{-1}) for 45 days.

For rooting, the exposure time of the explants to IBA was tested. For this purpose, 20 mg L^{-1} of IBA were used at 0, 24, 48, 72, and 96 hours. A completely randomized design was used, with 5 treatments, containing 12 replications for each *Pyrus comunnis* rootstock ('OHxF87' and Pyrodwarf). After the application of the treatments, the plants were exchanged from the tubes and cultivated in the culture medium; growth conditions were described for the multiplication protocol. After 45 days, the percentage of survival and rooting, the number of leaves and roots, shoot length and longest root length were evaluated.

The Shapiro-Wilk test was performed to assess data normality at a significance level of 5%. ANOVA was applied to the data that exhibited a normal distribution, comparing the means by the Scott-Knott test (5%). For data that did not have a normal distribution, the Kruskal-Wallis test was used, and the results were compared using the Nemenyi test. The software R was used for statistical analyses.

The 24-hour treatment provided 81,81% survival for 'OHxF87' rootstock. The same exposure time resulted in the highest survival rate (75%) for 'PDW' rootstock. Root formation did not occur in the absence of synthetic auxin (Figure 1).

Exposure time did not influence shoot length or the number of leaves in 'OHxF87' clone. The 24-, 72- and 96-hour treatments did not differ for length of the longest root. For the number of roots, there was no difference between treatments (Figure 1).

The 72-hour treatment led to the greatest growth in shoot length, differing only from the absence of IBA in 'PDW' clone. Exposure time did not influence number of leaves. Treatments 24 and 72 hours showed greater lengths of the longest root and number of roots, differing only from that with the absence of IBA (Figure 1).

In the literature, the protocols used for rooting selections of *P. communis* differ in terms of culture medium, type and concentration of plant growth regulator. For the selections 'OHxF' QL medium modified by Leblay yields satisfactory results in all stages of *in vitro* culture (SILVA et al., 2018). QL, ½MS and MS media for 'PDW' clone (LIZÁRRAGA et al., 2017; RUŽIĆ et al., 2011; SILVA et al., 2018). The forms and concentrations of IAA, IBA and naphthalene-acetic acid (NAA) ensure the rooting of cultivars and genotypes of different pear species (AYGUN & DUMANOGLU, 2015; BELL & REED, 2002; YANG et al., 2017), but with a very variable rooting rate, depending on the concentration of these plant growth regulators.

These results showed that the culture medium does not interfere with the rooting of these clones, but with the concentration of auxins. Auxins induce the formation of embryos from somatic cells, contributing to the formation and maintenance of the root apical meristem (TAIZ et al., 2017). In this study, exposure to IBA resulted in rooting rates above 80%, surpassing some results reported in the literature and confirming that *in vitro* rooting of clones occurs only in the presence of some type of treatment with a hormonal stimulus. Therefore, it can be concluded that an exposure time of 24 hours at 20 mg L^{-1} IBA was sufficient to promote 'OHxF87' and Pyrodwarf rootstocks' *in vitro* rooting.

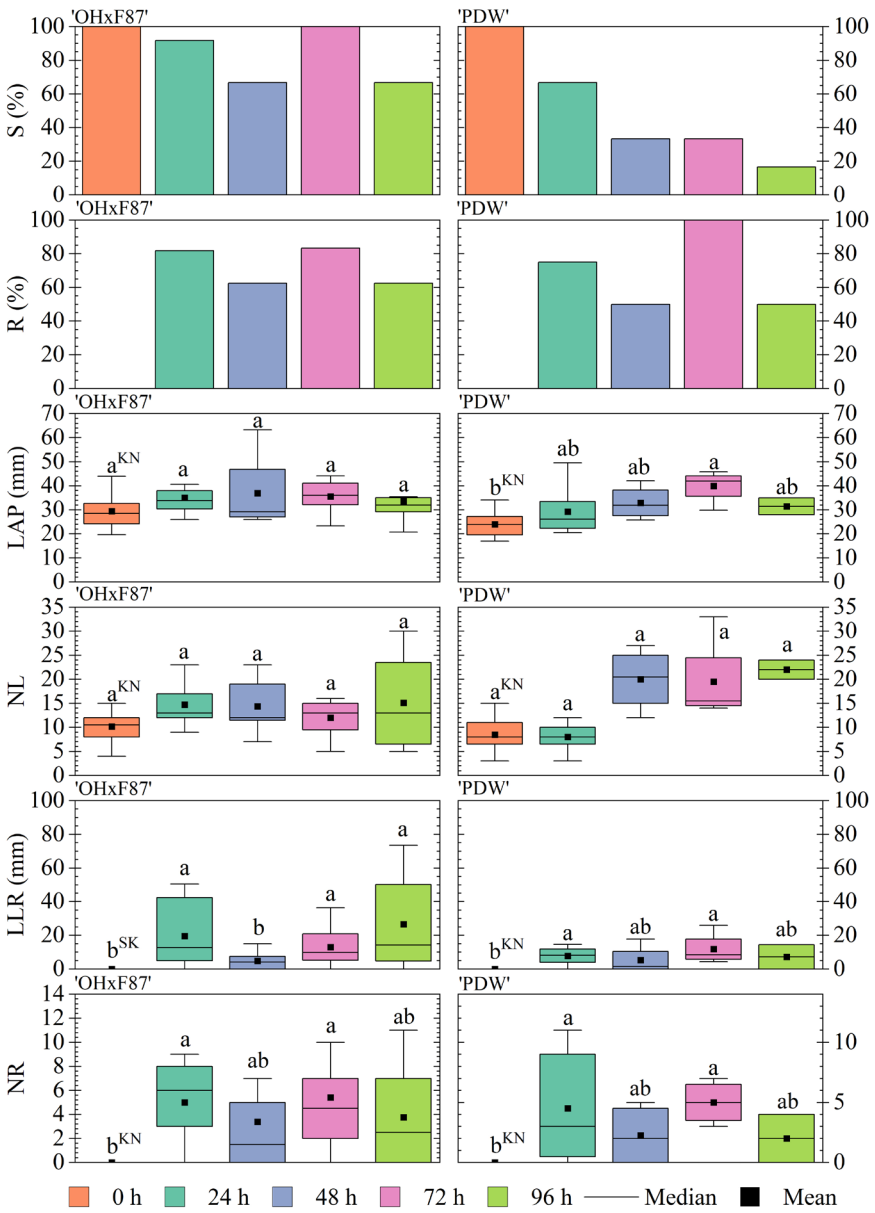

Figure 1 - Boxplot and statistical test of the rooting of 'OHxF87' and 'PDW' rootstocks exposed to synthetic auxin (IBA) for different times for 45 days of *in vitro* cultivation.

Variable: S - percentage of plant survival; R- percentage of rooting; LAP – shoot length, in mm; NL - number of leaves; LLR - length of longest root, in mm; NR - number of roots. Treatment: Exposure times of 0, 24, 48, 72, and 96 hours. Statistics: SK - means followed by the same letter do not differ by the Scott-Knott test at 5% significance; KN - means followed by the same letter do not differ by the Kruskal-Wallis-Nemenyi test at 5% significance.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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