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BIOLOGY

Micropropagation of the new apple rootstock 'G. 814'

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ABSTRACT: *International breeding programs launched new genetic material of apple rootstocks that in addition to precocity and great yield are resistant to major diseases and soil pests encountered in the largest apple producing regions in Brazil. Given this, there is a necessity for vegetative propagation of these materials for study and possible replacement of existing rootstocks. The objective was to adapt a micropropagation protocol for new apple rootstock 'G. 814'. In the multiplication phase were evaluated BAP concentrations: 0; 0.5; 1; 2 and 4mg L^{-1} and in the rooting phase were evaluated IBA concentrations: 0; 0.25; 0.50; 1; 1.5 and 2.5mg L^{-1}. These new results demonstrated that this new rootstock selection can be propagated with this tissue culture adapted protocol. For the successful in vitro propagation of apple rootstock 'G. 814' it is indicated the use of 1mg L^{-1} BAP at multiplication phase and 1.5mg L^{-1} IBA at rooting phase.*
Key words: *Malus sp., in vitro culture, plant growth regulators.*

Micropropagação do novo porta-enxerto de macieira 'G. 814'

RESUMO: *Programas de melhoramento internacional lançaram novos materiais genéticos de porta-enxertos de macieira que além de precoces e produtivos são resistentes as principais doenças e pragas de solo das maiores regiões produtoras de maçã no Brasil. Diante disto, existe a necessidade de propagação vegetativa destes materiais para estudos e possível substituição dos atuais porta-enxertos. O objetivo foi adaptar um protocolo de micropropagação para o novo porta-enxerto de macieira 'G. 814'. Na fase de multiplicação dos explantes foram avaliadas concentrações de BAP: 0; 0.5; 1; 2 e 4mg L^{-1} e na de enraizamento concentrações de AIB: 0; 0.25; 0.50; 1; 1.5 e 2.5mg L^{-1}. Esta seleção de porta-enxerto pode ser micropropagada com este protocolo adaptado. Para o sucesso na propagação in vitro do porta-enxerto de macieira 'G. 814'* é indicado o uso de 1mg L^{-1} *de BAP na fase de multiplicação e de 1.5mg L^{-1} de AIB na fase de enraizamento.*
Palavras-chave: *Malus sp., cultivo in vitro, fitorreguladores.*

The Brazilian apple production expanded significantly in the last two decades due to factors such as the production of modern varieties, rootstocks with phytosanitary quality and high-density orchards, increasing production around one million tons, where Santa Catarina State is responsible for 59% of national production (BITTENCOURT, 2011). Rootstock 'G. 814' is developed by the breeding program of Cornell-Geneva (Geneva® series), that state the most complete rootstocks in relation to high-density systems and resistant to major pests and soil diseases of apple orchards in Brazil (DENARDI et al., 2015), and according to PASA et al. (2016), the rootstock from this series looks very promising for the South region of Brazil. Thus, the micropropagation of adapted rootstocks to different apple cultivars is being widely studied today in order to obtain a commercial scale production and the possible replacement by new rootstocks, which requires the availability of large amount of propagation material.

According to the review made by DOBRÁNSZKI & SILVA (2010) there are a range of protocols that can be used for apple rootstocks, although, there is a strong genotype

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dependence regarding the salt composition of the best propagation media. The most regular media used for apples include MS, QL, WPM and DKW; therefore, the objective of this study was to adapt a micropropagation protocol for the new apple rootstock 'G. 814' aiming a larger scale production of this material.

This research consisted of two parallel experiments and was conducted in the years 2012 and 2013 in the Laboratory of Plant Micropropagation of Santa Catarina State University, located in Lages - SC. In the first experiment, nodal segments of 'G. 814' were used as explants with 2±0.5cm size, and the treatments were BAP concentrations (6-benzylaminopurine): 0; 0.5; 1; 2 and 4mg L^{-1}. In the second experiment, explants from the multiplication phase, previously cultured media in with 1mg L^{-1} of BAP, were used with 3±0.5cm size, and the treatments were IBA concentrations: 0; 0.25; 0.50; 1.0; 1.5 and 2.5mg L^{-1}.

In both studies, the culture media used was QL modified by LEBLAY et al. (1991), supplemented with 100mg L^{-1} of inositol, 30g L^{-1} of sucrose and 6g L^{-1} of agar, pH adjusted to 5.8 prior to addition of agar. Subsequently it was autoclaved at 121°C and 1.5atm for 20 minutes. After the inoculation of explants into the culture media, they were stored under controlled conditions of 27µmol $m^{-2}s^{-1}$ light intensity, photoperiod of 16 hours and temperature of 25±2°C.

The experimental design was completely randomized, with 6 replicants of 5 explants per treatment. After a period of 45 days, the variables analyzed in the multiplication phase were number of shoots, buds and leaves and length of shoots In the rooting phase were analyzed percentage and number of roots, length of roots and shoots, and the presence of callus. The data were submitted to analysis of variance and regression analysis using the program Sisvar.

During the *in vitro* multiplication there was no significant difference for the variables number of leaves and number of buds. JAMES & THURBON (2015) when working with 'M.9' rootstock observed that BAP concentrations of 1 and 2mg L^{-1} increased multiplication variables. In this study, for the rootstock 'G. 814', this was not an essential growth regulator to break apical dominance of shoots in order to provide a larger number of leaves and buds for the explants. For the variable length of shoots was adjusted a second-degree equation, where intermediate concentrations (between 0.5 and 3mg L^{-1}) had lower responses. It was reported that the treatment without BAP and concentrations above 3.5mg L^{-1} increased the length of explants, 11.1 and 9.7mm respectively (Figure 1b). Given the small difference reported between the absence and high concentration of this growth regulator in the length of shoots, it is justified the use of culture media without BAP, permitting a reduction in costs (CAMARGO et al., 2015).

For number of shoots, which is the most important variable in this stage, it was adjusted a second-degree equation, where low and high concentrations provided lower sprouting, and 1mg L^{-1} BAP (Figure 1a) had the best response (9.7 shoots). According to DOBRÁNSZKI & SILVA (2010), multiplication of apple explants depends on several factors: genotype; organic and inorganic compounds and plant growth regulators, and the cytokinin BAP is used commonly in the range of 0.5 to 5.0mg L^{-1} (GRATTAPAGLIA & MACHADO, 1998). The authors SANTA CATARINA (2001) concluded that for apple rootstock 'Selection 69' combining 0.5mg L^{-1} of BAP and GA3 promoted the greatest number of shoots. GENG et al. (2016) aiming the shoots and length increase of *in vitro* explants of different selections from the same Cornell-Geneva breeding program, 'G.30' and 'G.41', observed that the concentration 2.0mg L^{-1} of BAP were more effective to increase number of shoots, also being more effective than thidiazuron (TDZ) or zeatin (ZT). These results indicated that there is not an exact concentration of cytokinin, and for every selection, even from the same breeding program, a concentration must be adjusted.

In the study of *in vitro* rooting was observed significant differences for all variables, and all had a similar behavior, where concentrations above 1.5mg L^{-1} IBA provided a decrease in rooting percentage, number and length of roots. The Maximum Technical Efficiency (MTE) to the percentage of rooted plants (94%) was at 1.73mg L^{-1} concentration of IBA (Figure 2a). Higher number of roots (2.1) and length (6cm) were at 1.5 and 1.0mg L^{-1} concentrations (Figures 1b and 1c), respectively. According to CENTELLAS et al. (1999) several authors achieved good results using IBA between 1 and 3mg L^{-1} (5-15µM) for apple tree rooting. For the authors BHATTI & JHA

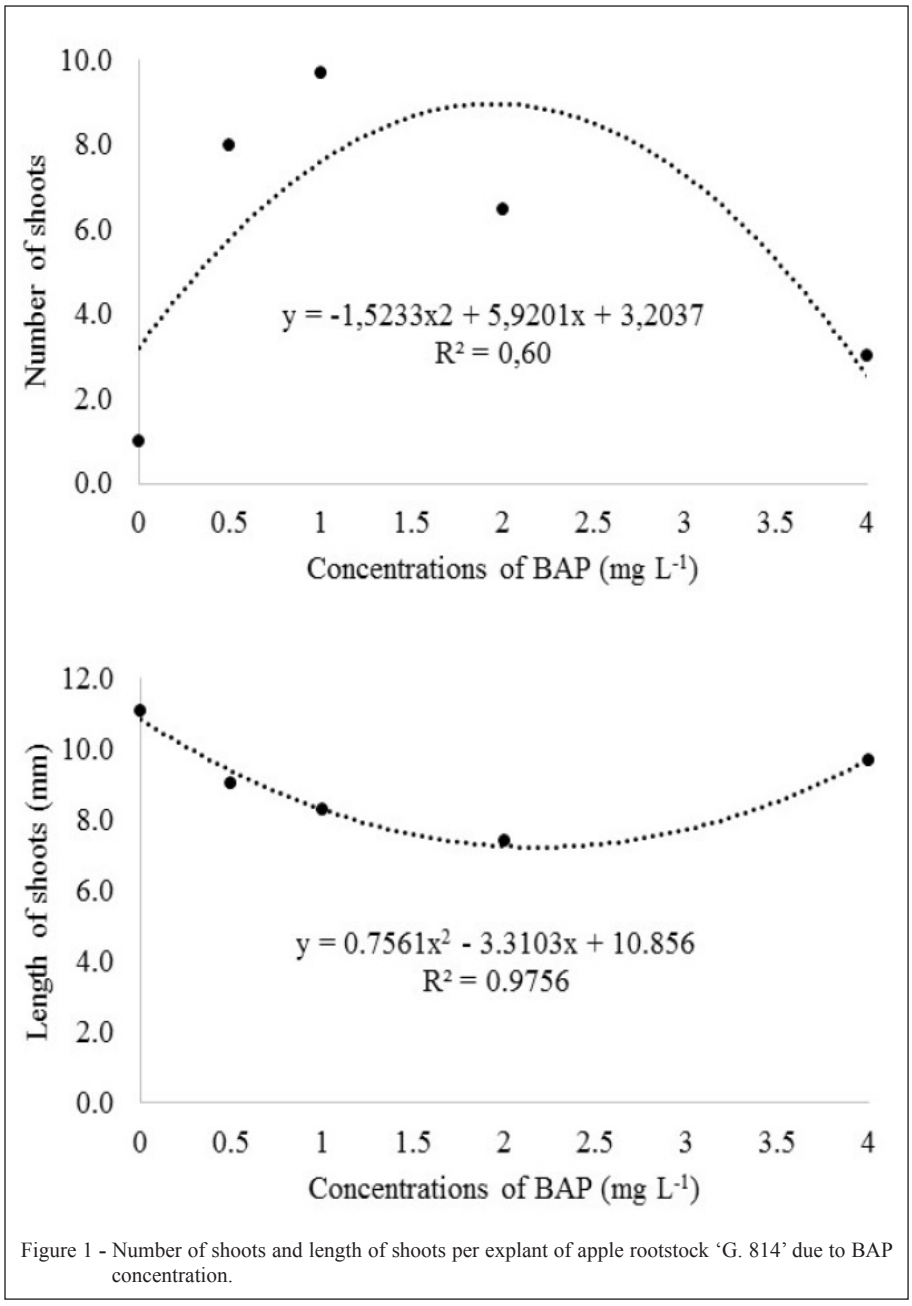

Figure 1 - Number of shoots and length of shoots per explant of apple rootstock 'G. 814' due to BAP concentration.

(2010), the auxin studied in this research (IBA) tends to be more favorable for the root system development of apple trees, and according to JONES & HATFIELD (2015) can increase the rooting proportion when combined with phenolic compounds. To the variable presence of callus (%) was observed a directly proportional relationship between occurrence of callus and IBA concentration, where higher amount of IBA in the media reflected on greater callus on the explants.

The results demonstrated that the new apple rootstock 'G. 814' can be successfully micropropagated with the adapted protocol presented, which indicated the use of 1mg L^{-1} BAP at *in vitro* multiplication phase and 1.5mg L^{-1} IBA at *in vitro* rooting phase.

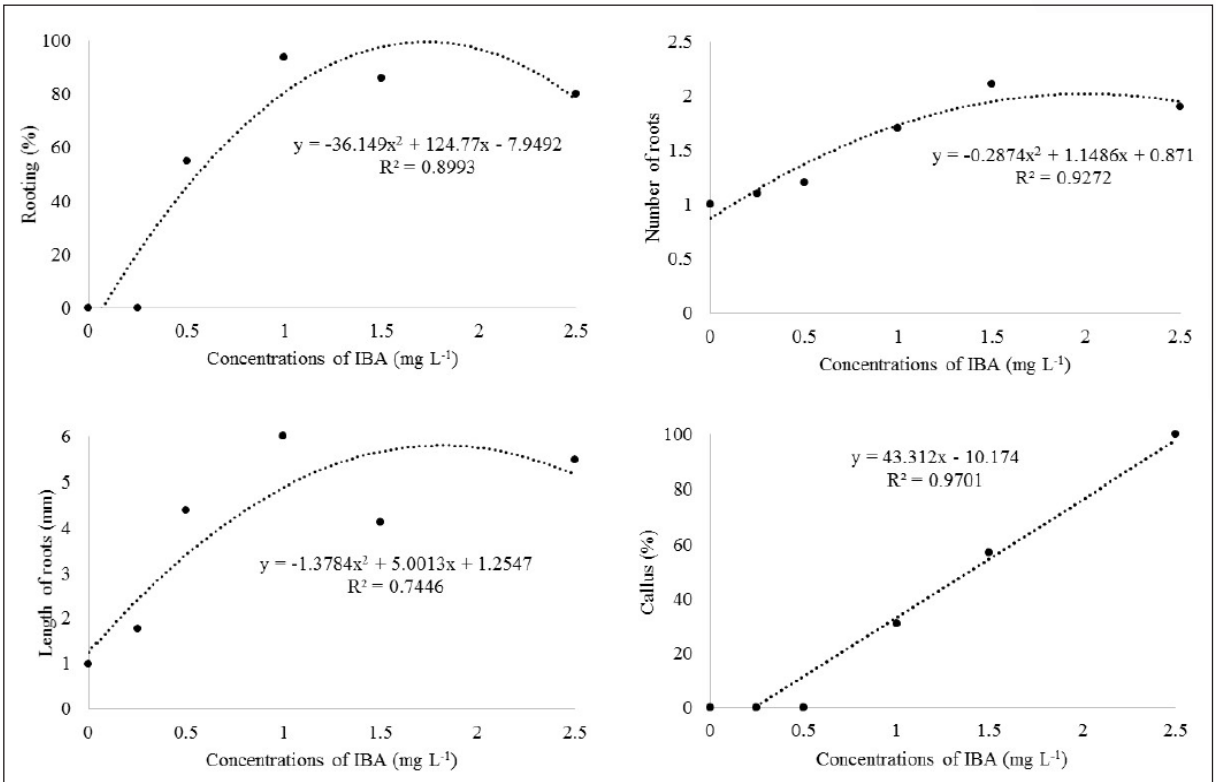

Figure 2 - Percentage of rooting, number of roots, length of roots and percentage of callus in explants of apple rootstock 'G. 814' due to IBA concentration.

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