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TRANSFORMAÇÃO GENÉTICA DE LARANJEIRAS APIRENAS COM USO DE TECIDO ADULTO

Jenifer A. P. **Corrêa[1];** Fábio Barufaldi De **Nadai[2]**; Rodrigo Rocha **Latado[3]**

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RESUMO – *Os carotenoides presentes nas plantas são extremamente importantes pois promovem a coloração das flores e frutos, protegem as clorofilas da foto-oxidação e são precursores do ácido abscísico. O β-caroteno e α-caroteno são encontrados principalmente em frutas e legumes e são percursores da vitamina A. O melhoramento clássico de citros é dificultado pelo ciclo juvenil longo, alta taxa de crescimento vegetativo e incapacidade de florescimento/frutificação no curto espaço de tempo. O método mais usual de transformação genética em citros envolve o uso de explantes originados de tecidos jovens, o que resulta na regeneração de plantas juvenis e prejudica a avaliação precoce dos transgenes. Este projeto envolve o uso da laranjeira doce X11, um mutante que possui plantas com florescimento precoce e capacidade de florescer em várias épocas do ano. Diante disso, o objetivo deste trabalho é obter plântulas transformadas geneticamente utilizando como explantes ovários imaturos da laranjeira X11, tecido considerado como adulto. Os ovários foram introduzidos em meio de cultura para formação de calos, seguido da transformação dos calos via co-cultivo dos mesmos com Agrobacterium tumefaciens. Esta cepa continha o vetor binário Gateway pK7GWIWG2(II) que induz plantas ao silenciamento do gene LCY-b2 alelo a. Em seguida tentou-se induzir à embriogênese somática em calos, seguido de formação e germinação dos embriões. A confirmação da obtenção de transgenes foi realizada por meio da técnica de PCR de amostras de folhas de plântulas regeneradas. De acordo com os resultados obtidos, pôde-se afirmar que houve variação na porcentagem dos calos embriogênicos obtidos (7,14 a 40%), nos diferentes tempos de co-cultivo testados (0, 24, 48 e 72h) e taxa de germinação cutura de*

1 Autor, Bolsista CNPq (PIBIC): Graduação em Biotecnologia, UFSCar, Araras-SP; jenifer.adriele@gmail.com.
2 Bolsista CAPES: Doutorando em Agricultura Tropical e Subtropial, CCSM – IAC, Cordeiróplis-SP.
3 Orientador: Pesquisador Científico, Centro de Citricultura Sylvio Moreira – IAC, Cordeiróplis-SP; rrlatado@gmail.com.

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embriões próxima de 1%, no experimento com 24 horas de co-cultivo. Até o presente momento nenhuma planta regenerada demostrou ser transgênica. Destaca-se, assim, a necessidade de otimizar o protocolo de transformação genética de calos obtidos por meio de cultura de óvulos imaturos.

Palavras-chaves: embriogênese somática, cultura de tecidos, melhoramento, carotenoides, C*itrus sinensis*.

ABSTRACT – Carotenoids are extremely important to plants because they promote flowers and fruits colors, protect chlorophylls from photooxidation and are precursors of abscisic acid. β-carotene and α-carotene are found mainly in fruits and vegetables and are precursors of vitamin A. Citrus improvement by crosses is hampered by long juvenile cycle, sterility and inability of seeded plants to flowering/fruiting in a short period of time. The most common method of genetic transformation in citrus involves the use of explants originating from young tissues (usually plantlets), which results in regeneration of juvenile plants and impairs the early evaluation of transgenes. This project involves the use of sweet orange X11, a mutant that has early flowering plants and the ability to bloom at various times of the year. Therefore, the objective of this work is to obtain genetically transformed seedlings using immature ovarian explants of sweet orange X11, tissue considered as adult. The ovaries were introduced into culture medium for callus formation, followed by callus transformation via co-ulture with Agrobacterium tumefaciens. This strain contained the binary Gateway vector pK7GWIWG2 (II) which induces plants to silencing LCY-b2 allele 'a' gene. Then, it was attempted to induce somatic embryogenesis in callus followed by embryo formation and germination. The confirmation of transgenes was performed through PCR technique of leaf samples from regenerated seedlings. According to obtained results, it was possible to affirm that there was variation in percentage of embryogenic callus formation (7.14 to 40%), in the different co-cultivation times tested (0, 24, 48 and 72h) and germination rate of 1% in the experiment with 24 hours of co-cultivation. To date, no regenerated plants have been shown to be transgenic. Thus, the need to optimize protocol of genetic transformation of callus obtained through immature ovule culture is emphasized.

1 Autor, Bolsista CNPq (PIBIC): Graduação em Biotecnologia, UFSCar, Araras-SP; jenifer.adriele@gmail.com.
2 Bolsista CAPES: Doutorando em Agricultura Tropical e Subtropial, CCSM – IAC, Cordeiróplis-SP.
3 Orientador: Pesquisador Científico, Centro de Citricultura Sylvio Moreira – IAC, Cordeiróplis-SP; rrlatado@gmail.com.

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Carotenoids are presents in plants and they are important, because promote the coloring of flowers and fruits, are precursors of abscisic acid, transfer solar energy and protect the chlorophylls from photo-oxidation. β-carotene and α-carotene are found mainly in fruits and vegetables and are precursors of vitamin A, which in turn is important for adequate human growth and differentiation of the tissues of several organs. Classical methods in citrus breeding is hard by the long juvenile cycle, high vegetative growth rate and flowering absence in short-term, and genetic transformation of young tissues is difficult by long-term phenotype evaluation. This project involves the use of a sweet orange named X11, which has short juvenile cycle, early flowering and ability to flowering at all seasons of the year. Therefore, the goal of this work is to obtain seedlings genetically modified using the ovary of X11 orange tree as adult tissue. The ovaries were introduced in callus induction culture medium followed by callus transformation, co-cultivation with Agrobacterium tumefaciens, with Gateway vector pK7GWIWG2 (II) into LCY-b2a gene silencing, followed for embryo formation and germination, followed by PCR of regenerated shoots. According to the results, it is possible to affirm there was a variation in the percentage of embryogenic callus (7,14-40%) in the different times of co-culture (0, 24, 48 and 72h) and the rate of 1% of embryo with 24 hours of co-cultivation, thus highlighting the need to further optimize the protocol of genetic transformation.

Keywords: s*omatic embryogenesis, tissue culture, breeding, carotenoids, Citrus sinensis*

1 Autor, Bolsista CNPq (PIBIC): Graduação em Biotecnologia, UFSCar, Araras-SP; jenifer.adriele@gmail.com.
2 Bolsista CAPES: Doutorando em Agricultura Tropical e Subtropial, CCSM – IAC, Cordeiróplis-SP.
3 Orientador: Pesquisador Científico, Centro de Citricultura Sylvio Moreira – IAC, Cordeiróplis-SP; rrlatado@gmail.com.