

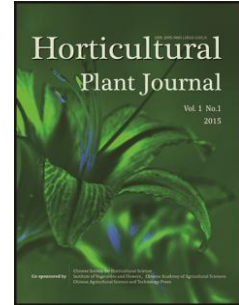
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Title: Mitochondrial Genome of Callus Protoplast Has a Role in Mesophyll Protoplast Regeneration in *Citrus*: Evidence From Transgenic GFP Somatic Homo-Fusion

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1 **Mitochondrial Genome of Callus Protoplast Has a Role in Mesophyll Protoplast**
2 **Regeneration in *Citrus*: Evidence from Transgenic GFP Somatic Homo-fusion**

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10 2468-0141 ©2017 Chinese Society for Horticultural Science (CSHS) and Institute of Vegetables and Flowers
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12
13 **Abstract**

14 Protoplast fusion has great potential in citrus improvement. Although citrus
15 mesophyll protoplasts usually cannot divide and regenerate, symmetric protoplast
16 fusion of embryogenic callus protoplast + mesophyll protoplast sometimes results in
17 the regeneration of mesophyll-parent-type cybrids. It suggested that mitochondrial
18 DNA (mtDNA) from protoplasts of embryogenic callus parent plays an important role
19 in stimulating division and regeneration of mesophyll protoplasts. Herein, somatic
20 fusion was conducted via electrofusion between callus protoplasts isolated from
21 Valencia orange [*Citrus sinensis* (L.) Osbeck] cell suspension cultures and transgenic
22 GFP-tagged mesophyll protoplasts from the same genotype, i.e. transgenic Valencia
23 orange plants containing the green fluorescent protein (GFP) gene, in an effort to
24 elucidate whether mtDNA of callus line could stimulate the division and regeneration
25 of mesophyll protoplasts from the same genotype. Two embryoids and one plantlet
26 with GFP expression were successfully obtained and subsequent ploidy analysis by
27 flow cytometry indicated that they were all diploids. The regenerated diploid
28 embryoids and plantlet with GFP expression could be considered as ‘cybrids’ with
29 mtDNA from the callus protoplasts of Valencia orange. The result indicated that citrus
30 mesophyll-parent-type cybrid regeneration needed the stimulation of mtDNA from

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