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A protocol for the *in vitro* propagation and polyploidization of an interspecific hybrid of *Glandularia* (*G. peruviana* × *G. scrobiculata*)

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ABSTRACT

The genus *Glandularia* (Verbenaceae) has more than 50 species and it holds great ornamental potential due to its colorful flowers, long flowering period and low water requirements. Since the floriculture market avidly seeks novelties, increasing the diversity within the genus would increase its commercial value. Some of the traits that should be improved in these species are their architecture and the size and colors of the flowers and leaves. In this sense, obtaining polyploid individuals is an interesting strategy to achieve this objective. In this paper a hybrid of *Glandularia peruviana* × *Glandularia scrobiculata* was cultured under in vitro conditions. Different plant growth regulators and their combinations were tested to obtain appropriate multiplication rates. The best results were obtained with the combination of 6.6 μM thidiazuron/0.03 μM α-naphthalenacetic acid, with a multiplication rate of 19 shoots per explant. The plantlets were first rooted and then acclimatized by transferal to an 8.0 cm diameter pot containing Growing mix®, and maintenance inside a humidity chamber. For poliploydization, the explants were exposed to colchicine in concentrations of 0.001% and 0.01% for 24 and 48 h. The 40 recovered plants were characterized according to their DNA content. There were 21 diploids, 14 solid tetraploids, 1 solid octoploid, 3 chimera tetraploids and 1 chimera octoploid. Phenotypically, the size of the flowers, inflorescences, pollen grains and stomata were significantly larger in polyploid individuals. Surprisingly, stem diameter, leaf size and color intensity of the leaves and flowers were not significantly different between diploid and tetraploid individuals. Due to the size of the inflorescences, the tetraploid individuals are a promising starting material for a *Glandularia* breeding program.

1. Introduction

The genus *Glandularia* (Verbenaceae), comprises approximately 50 species of herbaceous annual and perennial plants (Zuloaga and Morrone, 1999). These species are found in temperate and subtropical regions of North and South America. In Argentina, they are common in the provinces of Buenos Aires, Entre Ríos, Corrientes, Formosa and Santa Fe (Peralta and Mulgura, 2011). *Glandularia* has important ornamental potential due, mainly, to its attractive flowers, which come in a wide variety of colors (white, red, pink, lilac or violet). Its long blooming period and low water requirements are also important features. Some of the members of this genus are erect, while others have creeping growth habits (Zuloaga and Morrone, 1999). Since the floriculture market avidly seeks novelties, increasing the diversity within the genus would increase its commercial value (Heywood, 2003).

Several objectives can be achieved when native plant germplasm with ornamental potential is used sustainably. For instance, it can provide the floriculture market with the novelties it so avidly seeks (Chandler and Tanaka, 2007; Rout et al., 2006). In this context, biotechnology offers a wide range of powerful tools for germplasm development. Plant tissue culture is based on the totipotency of plant cells and is one of the main tools employed in the floriculture industry because it allows the massive propagation of the selected species. Furthermore, it can also yield valuable insights into the physiology of the species. It is also the starting

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point for the application of other biotechnological techniques that can contribute to plant breeding (Escandón et al., 2010).

There are few reports regarding the application of biotechnological strategies for germplasm breeding of *Glandularia*, and this highlights the relevance of this research. In fact, the only references found regarding the *in vitro* propagation of this genus were the previous works of this group on *Glandularia peruviana* (Iannicelli et al., 2010a,b; 2012) and the research carried out by Marino et al. (2003) and Ponce et al. (2010).

Polyploidization has played a leading role in the evolutionary development of many plant species (Chen and Zhongfu, 2006). In recent years, synthetic ploidy breeding has also made an important contribution to crop domestication and to the development of new cultivars, *e.g.*, creating crops with specific traits such as larger flowers and fruits (Yang et al., 2011). This methodology has been historically used, especially in ornamental crops, to obtain new morphological characteristics or to overcome interspecific crossing barriers (Eeckhaut et al., 2006; Horn, 2002). However, there is still limited knowledge concerning all the effects that polyploidization can cause in plant processes. Obtaining autoploid individuals by application of the mutagenic agent colchicine increases the variability of phenotypes by altering the size, shape and color of leaves, flowers and stems and it can also affect plant architecture (Eeckhaut et al., 2004; Escandón et al., 2005, 2006, 2007; Dhooghe et al., 2011).

In this paper, we report a protocol to obtain new phenotypes of *Glandularia* from an artificial hybrid between *G. peruviana* (Griseb)

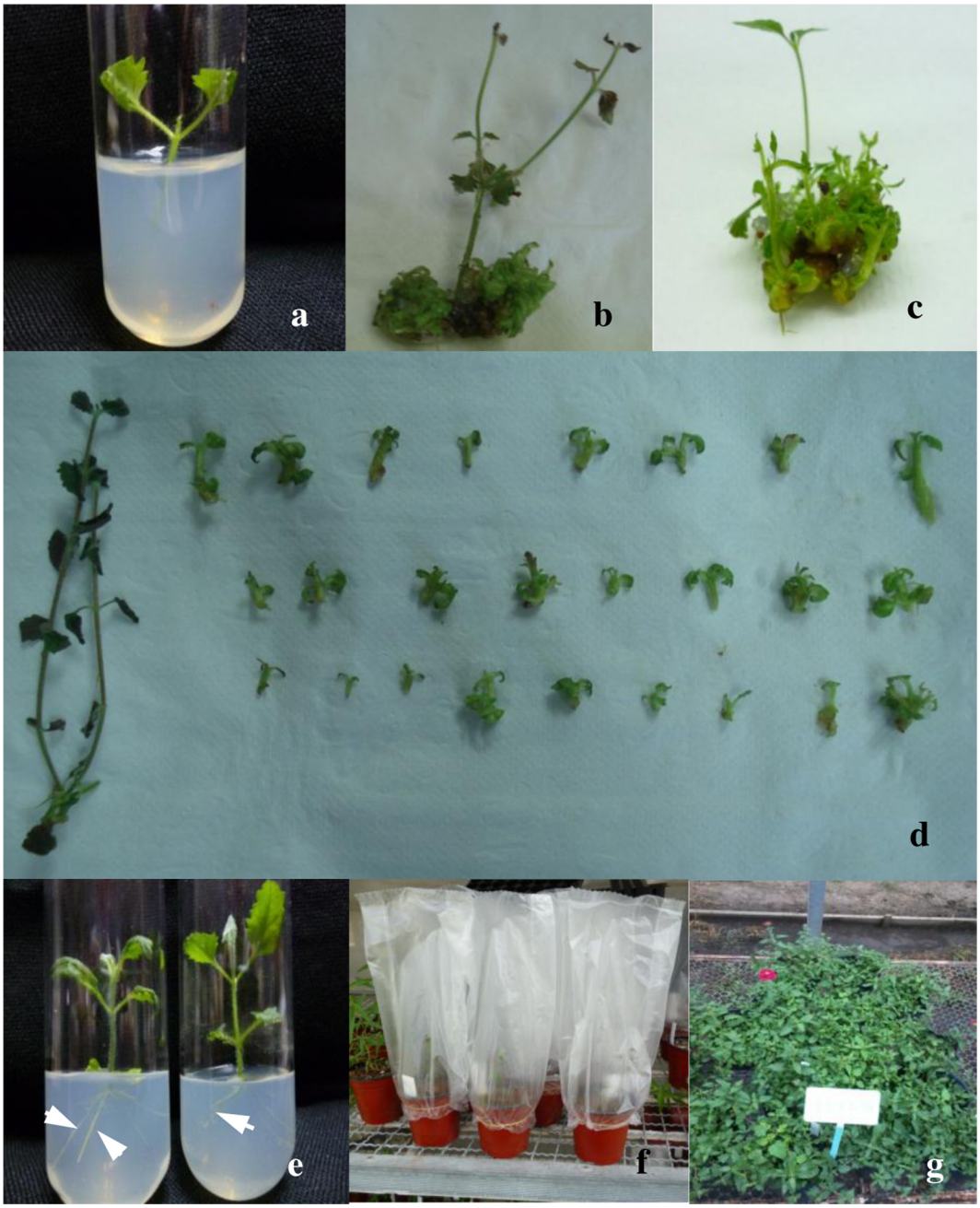

Fig. 1. *In vitro* development of *Glandularia* hybrid. The explants were single-node segments. (a) Early stage of *in vitro* culture. (b) Explant cultured on WPM + 6.6 μM TDZ (c) Explant cultured on WPM + 6.6 μM TDZ/0.03 μM NAA. In both (b and c), shoot development from the base of the explant was observed after 30 days. (d) 25 shoots recovered from one explant cultured on WPM + 6.6 μM TDZ/0.03 μM NAA. (e) Plantlets during de rooting step (roots arrowed). (f) Acclimatization phase. (g) Viable *ex vitro* plants growing under standard green house conditions.