Hormone-injected leaf cutting, a new efficient *in vivo* multiplication protocol for two succulent plants

Xiaodan Xu¹ and Wei Zheng²,*

¹ Faculty of Art and Communication
Kunming University of Science and Technology
Kunming 650500
China

² Faculty of Architecture and City Planning
Kunming University of Science and Technology
Kunming 650500
China

* Corresponding author:
E-mail: 57280234@qq.com.
Abstract: This study aimed to establish a simple and efficient *in vivo* multiplication protocol by leaf cutting to satisfy the supply of young succulent ornamentals *Pachyveria pachyoides* and *Sedum morganianum*. The regenerability of leaves injected with 6-benzylaminopurine (BAP) and α-naphthalene acetic acid (NAA) *in vivo* were tested with common leaf cutting as control. Results showed a 100% shoot induction frequency using hormone-injected methods for the two species. The number of shoots per leaf of 4.0 or 6.0 mg l\(^{-1}\) BAP and 0.1 mg l\(^{-1}\) NAA injected *in vivo* (5.08-5.14 in *P. pachyoides*, and 6.22-6.74 for *S. morganianum*) were significantly greater than that of the other treatments. Since the hormone-injected leaf cutting needs no aseptic operation which is necessary for *in vitro* multiplication, it is simple for the commercial production of the two species. The new *in vivo* propagation method would be of great interest for growers and breeders of succulent plants.

Keywords: *Pachyveria pachyoides*, *Sedum morganianum*, bud multiplication, direct organogenesis
1. Introduction

*In vitro* proliferation is currently applied to propagate many ornamental plants (Jaykuma et al., 2013; Ozel et al., 2008; Chikkala et al., 2009), but commercial production requires the use of cost-effective *in vivo* multiplication.

Succulent plants are characterized by thick and fleshy parts to retain water in arid climates or soil conditions (Nyffeler and Eggi, 2010). They are grown as ornamental plants because of their striking and unusual appearance. The family Crassulaceae is a large group of succulent plants (Griffiths, 2013) with several genera, such as *Echeveria* (Borys and Leszczynska-Borys, 2013), *Sedum* (Sendo et al., 2007), *Kalanchoe* (Heiko et al., 2014), and *Pachyveria*; in this family of plants, species and progenies vary in terms of the size of their rosette leaves, color, fatty leaves, and length of vegetative and reproductive organs, as well as ease of cultivation (Rafael et al., 2013).

Leaf cutting is one of the main propagation methods for succulent plants. One (seldom two) bud and multiple roots can be directly germinated from petioles via leaf cutting. Compared with that of hormone-supplemented *in vitro* propagation, the multiplication time of common leaf cutting is very low.

Here, we speculate that better *in vivo* multiplication via bud formation can be achieved if the fatty leaves were considered as the basic MS medium (Murashige and Skoog, 1962) and the hormones were injected into the mesophyll cells of these leaves. However, no detailed reports have been presented regarding hormone treatment in succulent plants *in vivo.*
We aimed to establish a simple and efficient *in vivo* proliferation protocol for succulent plants by using leaf cutting of *Pachyveria pachytoides* and *Sedum morganianum*, which are members of the family Crassulaceae endemic to Mexico and cultivated worldwide as ornamental plants (José et al., 2010), to verify our hypothesis. The research would be useful for commercial production and further research of succulent plants.

2. Materials and Methods

2.1 Materials

Plants of *P. pachytoides* and *S. morganianum* were bought from Dounan Flower Market, Kunming, Yunnan Province, China and cultivated in a greenhouse in Kunming University of Science and Technology.

2.2 Direct organogenesis from hormone-injected in vivo leaves

The mature leaves of the two species were excised from their mother plants and dried in the shade for 3 d. The average leaf volume (3.0 ml for *P. pachytoides* and 0.6 ml for *S. morganianum*) and its solution-absorbing capacity (0.10 ml for *P. pachytoides* and 0.02 ml for *S. morganianum*) were measured. Afterward, the hormone concentrations were calculated (60.0, 120.0, and 180.0 mg l\(^{-1}\) BAP; 3.0 mg l\(^{-1}\) NAA) to obtain the final hormone concentration of 2.0, 4.0, and 6.0 mg l\(^{-1}\) BAP and 0.1 mg l\(^{-1}\) NAA in the leaves after injection. Injection was made near the petiole and deep into the mid-blade. With the adaxial side facing upward, the injected
leaves were then planted in a mixture of peat, vermiculite, and perlite (1:1:1) in a Petri dish.

2.3 Experimental design and statistical analyses

All of the experiments were conducted in three repetitions (20 explants per repetition) by a randomized complete block design. Common leaf cutting without hormone treatment was performed as control. All of the cultures were maintained in a growth chamber at 20 °C to 25 °C (Nishida et al., 2009) under cool, white fluorescent lamps (45 \( \mu \)mol s\(^{-1}\) m\(^{-2}\)) with 14 h photoperiod. The number of days to shoot or root initiation for each treatment was recorded. The average number of adventitious shoots or roots induced per leaf and the induction frequency were measured after 30 d of culture.

Data in the form of percentages were subjected to arcsine transformation whereas non-transformed data are presented in tables. Data were analyzed by the analysis of variance (ANOVA) and Duncan’s multiple range test with a 0.05 confidence interval.

3. Results

Adventitious shoots and roots originated directly from parenchyma cells near the vascular bundle sheath of \textit{in vivo} and \textit{in vitro} propagated leaves of \textit{P. pachyoides} and \textit{S. morganianum}. The buds of \textit{P. pachyoides} and \textit{S. morganianum} were initiated
around 7 and 15 d after propagation, respectively. In addition, these two succulent leaves developed roots around 18 d after propagated.

In the culture of two succulent plants, the shoot induction frequency was 100% for all the treatments (Table 1). The number of shoots per leaf (5.08-5.14 in *P. pachytoides*, and 6.22-6.74 for *S. morganianum*) of 4.0 or 6.0 mg l\(^{-1}\) BAP and 0.1 mg l\(^{-1}\) NAA injected *in vivo* was significantly greater than that of the other treatments (Figure 1). While, the root induction frequency by hormone-injected methods was lower than that of common cutting (100%). In addition, the number of roots decreased with the increasing of BAP concentration. This result indicated that a higher concentration of BAP could induce bud formation than root formation.

4. Discussion

These succulent ornamental plants can be sold in the market as small plants (1 cm to 3 cm in height), even without roots. Therefore, this study mainly aimed to promote multiple bud induction and proliferation rather than root formation. In this experiment, 4.0:0.1 BAP:NAA hormone injected leaf cutting could be used for multiplication of *P. pachytoides* and *S. morganianum* because the induction of shoots was the highest in this treatment, as well as the merits of cheap and simple.

Compared with *in vitro* propagation, hormone-injected *in vivo* propagation did not require aseptic operation, thereby reducing workload and production cost. Therefore, hormone-injected *in vivo* leaf cutting is an effective method for the
commercial production of *P. pachyoides* and *S. morganianum*, even other succulent plants.

Further studies could be conducted to exploit the potential of mutation and genetic transformation for the improvement of succulent ornamentals by using this new hormone-injected *in vivo* propagation.

References


Acknowledgments

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Figure legends

**Fig. 1.** Direct organogenesis from the leaves of *Pachyveria pachytoides* (A, B) and *Sedum morganianum* (C, D) after 30 days of propagation. (A, C) Common leaf cutting; (B, D) hormone (4.0 mg l$^{-1}$ BAP + 0.1 mg l$^{-1}$ NAA)-injected *in vivo* leaves.
Table 1 Effect of different method on direct organogenesis of *Pachyveria pachytoides* and *Sedum morganianum*.

<table>
<thead>
<tr>
<th>Species</th>
<th>BAP + NAA (mg l⁻¹)</th>
<th>Shoot induction (%)</th>
<th>No. of shoots/leaf</th>
<th>Root induction (%)</th>
<th>No. of roots/leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pachyveria pachytoides</em></td>
<td>0 + 0</td>
<td>100.00 a</td>
<td>1.16 ± 0.12 c</td>
<td>100.00 a</td>
<td>8.13 ± 0.92 a</td>
</tr>
<tr>
<td></td>
<td>2 + 0.1</td>
<td>100.00 a</td>
<td>3.79 ± 0.40 b</td>
<td>92.67 a</td>
<td>1.05 ± 0.13 b</td>
</tr>
<tr>
<td></td>
<td>4 + 0.1</td>
<td>100.00 a</td>
<td>5.14 ± 0.36 a</td>
<td>33.65 ab</td>
<td>0.44 ± 0.03 c</td>
</tr>
<tr>
<td></td>
<td>6 + 0.1</td>
<td>100.00 a</td>
<td>5.08 ± 0.41 a</td>
<td>10.24 b</td>
<td>0.12 ± 0.01 c</td>
</tr>
<tr>
<td><em>Sedum morganianum</em></td>
<td>0 + 0</td>
<td>100.00 a</td>
<td>1.37 ± 0.15 c</td>
<td>100.00 a</td>
<td>5.62 ± 0.78 a</td>
</tr>
<tr>
<td></td>
<td>2 + 0.1</td>
<td>100.00 a</td>
<td>3.93 ± 0.41 b</td>
<td>90.00 a</td>
<td>2.51 ± 0.32 b</td>
</tr>
<tr>
<td></td>
<td>4 + 0.1</td>
<td>100.00 a</td>
<td>6.74 ± 0.58 a</td>
<td>83.67 a</td>
<td>1.90 ± 0.20 c</td>
</tr>
<tr>
<td></td>
<td>6 + 0.1</td>
<td>100.00 a</td>
<td>6.22 ± 0.61 a</td>
<td>82.10 a</td>
<td>1.82 ± 0.21 c</td>
</tr>
</tbody>
</table>

Values within a column for each species followed by the same letter are not significantly different at *p*=0.05 by Duncan’s multiple range test.