Micropropagation of *Lewisia cotyledon* using Axillary Buds from Flower Peduncles

Mary W George and Robert R Tripepi |

ABSTRACT

Lewisia cotyledon (S. Wats.) B.L. Robins. (Portulacaceae), a perennial native to the mountainous areas of the western US, was micropropagated using the lower axillary buds from flower peduncles. Successful establishment in tissue culture was genotype dependent. Driver Kuniyuki Walnut medium (DKW) supplemented with 3.5 µM 6-benzylaminopurine (BA) appeared to be a better basal medium for increasing and maintaining *in vitro* rosettes than either Murashige and Skoog medium (MS) or Woody Plant medium (WPM) supplemented with the same BA concentration, but this response was genotype dependent. Placing rosettes on MS medium supplemented with 9.8 µM indole-3-butyric acid (IBA) for 12 wk resulted in 100% rooting with an average of 16 roots per rosette. Rooted rosettes were successfully transferred to *ex vitro* culture and were phenotypically normal. Micropropagation of lewisia will enable growers to produce large numbers of plants rapidly for the home landscape.

KEY WORDS

tissue culture, Driver Kuniyuki Walnut medium, Murashige and Skoog medium, Woody Plant medium, indole-3-butyric acid, 6-benzylaminopurine, Portulacaceae

NOMENCLATURE

USDA NRCS (2002)

Lewisia cotyledon, propagated by tissue culture, flowering in the senior author's rock garden in Moscow, Idaho.

Lewisias are fleshy herbaceous perennial plants that are native to the mountainous areas of the western US. The growth habit of most species is a rosette that is 8 to 30 cm (3 to 12 in) in diameter (Mostul 1995), making them ideal for small gardens, rock gardens, and pot culture. The most popular species is *Lewisia cotyledon* (S. Wats.) B.L. Robins. (Portulacaceae) because of its outstanding flower display and ease of growth (Mostul 1995).

Lewisia species usually are propagated by seeds (Bailey and Bailey 1976), but germination can be sporadic (Davidson 2000). Lewisia species also can be asexually propagated by rooting the small offset rosettes (Davidson 2000), but this method produces only a small number of new plants over time. Micropropagation, a tissue culture procedure that allows rapid multiplication of a plant over a short period of time, could enable lewisia growers to produce enough plants to market them on a large scale. The process of micropropagation involves: 1) establishment of aseptic cultures through the selection of suitable explants, their surface disinfestations, and transfer to a nutrient medium; 2) multiplication of shoots that develop from the explants; 3) transfer of shoots to a rooting medium; and 4) transfer of rooted shoots to an *ex vitro* environment. Our study objective was to develop a rapid and efficient micropropagation procedure for *Lewisia cotyledon.*

MATERIALS AND METHODS

Fifteen *Lewisia cotyledon* stock plants were obtained from Rare Plant Research (Portland, Oregon) and kept in 3-l (1-gal) pots in a greenhouse at the University of Idaho, Moscow, Idaho. The growing conditions consisted of 27 °C (81 °F) daytime and 15 °C (59 °F) nighttime temperatures under a natural photoperiod. These plants provided flower peduncles for the following experiments.

Flower peduncle sections with 2 to 3 axillary buds were surface disinfested for 20 min in a 10% bleach solution containing about 4 drops of Tween 20 per liter (0.26 gal), then rinsed 3 times in sterile distilled water. These explants were placed in 120-ml (3.6-oz) baby food jars fitted with Magenta-B caps containing 25 ml (0.75 oz) of medium. Explants were placed immediately in a Hoffman S30G germinator at a constant 25 °C (77 °F) with a 16-h photoperiod and an average 40 µmol/(m^2 • s) photosynthetically active radiation.

The basal media used in the following experiments were: 1) Murashige and Skoog medium (MS) (Murashige and Skoog 1962); 2) Woody Plant medium (WPM) (Lloyd and McCown 1980); and 3) Driver Kuniyuki Walnut medium (DKW) (McGranahan and others 1987) (Table 1). Plant growth regulators used in the experiments were added before autoclaving. The pH for each medium was adjusted to 5.6 after adding plant growth regulators but before autoclaving. Media were autoclaved for 20 min at 121 °C (250 °F) and 98 kPa (15 lb/in).

Shoot Initiation and Multiplication from Flower Peduncles

The regeneration medium used for the axillary buds from the flower peduncles was either MS or WPM supplemented with either 6-benzylaminopurine (BA) (0, 4.4, or 8.9 µM) or thidiazuron (TDZ) (0, 0.5, or 0.9 µM). Surface disinfested nodal sections were placed on the media as they became available during flowering over a 2-mo period. If a bud produced a rosette of leaves, it was removed and placed on MS with 3.5 µM BA. Rosettes were transferred to fresh medium about every 4 wk. The total number of rosettes, newly formed and original propagules, was recorded after each transfer. After 16 mo, enough rosettes had been produced to test the effect of 3 different basal media on multiplication rate of lewisia rosettes. The media were MS, WPM, or DKW supplemented with 3.5 µM BA. Each medium treatment started with 40 rosettes (4 rosettes per jar). Rosettes were transferred to fresh medium every 4 wk. Total num-

TABLE 1

Formulations of the media used in the experiments in this study.

Compound	MS medium	DKW medium	WPM
Basal salts [a] (g/l)	4.3	5.2	2.3
Thiamine-HCL (mg/l)	2.0	1.0	1.0
Pyridoxine-HCL (mg/l)	1.0	0.5	0.5
Nicotinic acid (mg/l)	1.0	none	0.5
Glycine (mg/l)	4.0	2.0	2.0
myo-inositol (mg/l)	100.0	100.0	100.0
Sucrose (g/l)	30.0	30.0	20.0
Agar (g/l)	8.0	8.0	8.0

[a] Purchased from PhytoTechnology Laboratories, LLC, Shawnee Mission, Kansas; catalog numbers M524, D190, and L154 for MS, DKW, and WPM, respectively.

ber of rosettes, both new ones and the original propagules, was recorded after each transfer. These cultures were maintained in the Hoffman germinator at 25 °C (77 °F) with a 16-h photoperiod .

In Vitro Rooting of Rosettes

Rosettes of several genotypes were used in the *in vitro* rooting experiments. Indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), or ∝-naphthaleneacetic acid (NAA) were added to MS medium at 0, 0.5, 1.0, 1.5, or 2.0 µM concentrations. Four rosettes were placed in each jar; 5 jars were used per auxin treatment. Rosettes were maintained in the Hoffman germinator at a constant 25 °C (77 °F) with a 16-h photoperiod. At the end of 8 wk, rosettes were removed, and the number of rosettes that formed roots and numbers of roots formed on each rosette were counted. Rooted rosettes were planted in 10-cm (4-in) pots containing a soilless mix of 5 peat:4 perlite:3 coarse sand (v:v:v), 4 rosettes to a pot. Two-thirds of the pots were individually covered with plastic wrap held in place with a rubber band around the pot for 1 wk. The remaining third were left uncovered. Potted rosettes were placed in a Conviron E8 growth chamber at 25 °C (77 °F) with a 16-h photoperiod.

In a second experiment, we tested the effects of higher IBA concentrations and an additional 4-wk exposure to IBA in the *in vitro* rooting medium. Murashige and Skoog medium was supplemented with IBA at 0, 2.5, 4.9, 7.4, or 9.8 µM. Four rosettes were placed in each jar, and 5 jars were used per IBA concentration. After 8 wk, while still in the jars, the number of rosettes that formed roots was counted. At the end of 12 wk, rosettes were removed from culture and the number of rosettes that formed roots and the number of roots formed on each rosette were counted.

Only data collected from the rooting experiments were statistically analyzed. Percentages of rosettes that rooted were analyzed using a generalized linear model assuming a binomial distribution (rosettes rooted or failed to root) with a logit link function (Proc Genmod; SAS Institute 1999). Differences in the average number of roots formed among auxin treatments were analyzed using a generalized linear model assuming a Poisson distribution with a log link function.

TABLE 2

Effect of plant genotype, media, and plant growth regulators (PGR) on the initial number of rosettes formed.

Stock plant identification number	Media [a]	PGR [b]	Concentration (µM)	Number of nodal sections	Number of initial rosettes
3	MS	TDZ	0.0	1	0
			0.5	2	2
	WPM	TDZ	0.5	1	0
			0.9	2	0
4	WPM	TDZ	0.0	4	2
			0.9	4	10
Several stock plants	WPM	BA	0.0	1	3
			4.4	6	12
			8.9	3	8

[a] Media used were either MS (Murashige and Skoog) or WPM (Woody Plant medium).
[b] Plant growth regulators used were either TDZ (thidiazuron) or BA (6-benzylaminopurine).

RESULTS AND DISCUSSION

Shoot Initiation and Multiplication from Flower Peduncles

Because stock plants bloomed at different times and flowers were being produced asynchronously, completing the planned flower peduncle axillary bud experiments in a statistically relevant manner was not possible. Over a 2- to 3-mo period, 25 flower peduncles became available and the nodal sections were placed on various media (Table 2). Enough nodal sections were available from plant-3 and plant-4 to track the responses of these plants separately. Nodal sections contained 2 to 3 axillary buds. The nodes on the lower part of the flower peduncles produced new rosettes more often than the nodes found closer to the flower umbels. These latter nodes usually produced a flower. None of the jars with nodes from peduncles developed contamination. Contamination was a problem in previous micropropagation attempts of lewisia by others (Davidson 2000).

During the initiation phase, genotype affected axillary bud growth as plant-3 produced only 2 initial rosettes from 6 nodal sections, whereas plant-4 produced 12 initial rosettes from 8 nodal sections (Table 2). Rosette initiation from the axillary buds was apparently unaffected by media or plant growth regulators.

During the multiplication phase, genotype also affected multiplication rates. After 9 mo, all rosettes from plant-3 had died (Table 3), but rosettes from plant-4 increased 20X. For other plants, large numbers of rosettes were produced from a few axillary buds within a year (Table 3). By the second or third month in tissue culture, the number of rosettes almost doubled and continued to double for the next 2 months. After about 6 mo in culture, the numbers of rosettes continued to increase but at a slower rate.

Basal medium affected the multiplication rates, but response to basal salts

TABLE 3

Number of rosettes (newly formed and original propagules) after each monthly subculture by plant genotype. Explants were maintained on MS medium supplemented with 3.5 µM BA.

Stock plant identification number	Months in culture 0	1	2	3	4	5	6	7	8	9	10	11	12
2	NA	NA	NA	NA	87 [a]	42	77	152 [b]	48	81	163	147	134
3	2	2	2	3	4	7	6	6	5	4	0	0	0
4	7	7	15	39	28	59	113	105	111	142	177	180	221
Several stock plants	13	13	24	52	125	229	482	506 [c]	412	518	626	588	539

[a] 45 rosettes removed for rooting experiment. [b] 70 rosettes removed for rooting experiment. [c] 64 rosettes removed for rooting experiment.

was genotype specific. After being maintained on MS medium supplemented with 3.5 µM BA for 16 mo, rosettes from plant-4 and several other genotypes were placed on MS, DKW, or WPM containing 3.5 µM BA. After 6 mo on these 3 media, the total number of plant-4 rosettes on DKW had increased 6X, whereas rosette numbers on MS or WPM remained the same or decreased slightly (Figure 1A). However, multiplication of the mix of genotypes was unaffected by basal medium with rosettes on DKW medium responding similarly as those on MS or WPM media (Figure 1B). Rosette numbers initially increased on all media, then gradually declined to less than the initial number of rosettes. While MS and DKW are similar in ionic concentration (twice that of WPM), DKW has 3X the calcium as MS or WPM. Calcium deficiency results in stunted shoots and dieback of metabolically active tissues, such as growing apices of shoot cultures (Sha and others 1985). The higher calcium level of DKW may have promoted the active growth of new forming shoot meristems for plant-4.

Two abnormalities seen during the rosette multiplication phase were rosettes without central growing points or rosettes with multiple growing points. These multiple growing points

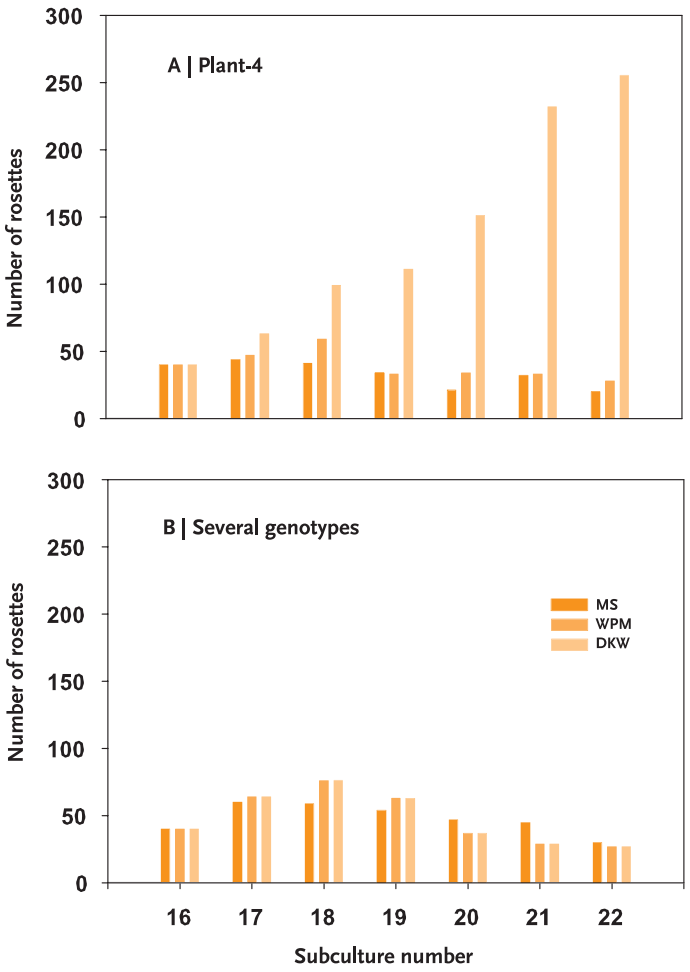

Figure 1. The effects of basal media on the numbers of rosettes formed after each monthly transfer to fresh medium: A) plant-4 only; B) mix of genotypes excluding plant-4. Numbers of rosettes include both newly formed rosettes and original propagules.

formed in a line, making division into a rosette difficult as compared to typical offset formation on the main rosette.

High temperature affected rosette growth. For 2 wk, the temperature in the tissue culture room where some of the cultures were kept was over 30 °C (86 °F) because of a malfunctioning air conditioner. These rosettes either bleached out or vitrified; many of the growing points died. The bleached rosettes recovered when they were returned to 25 °C (77 °F).

In Vitro Rooting of Rosettes

Neither the type of auxin nor concentration significantly affected the percentage of rosettes that rooted *in vitro* (Table 4). Rooting percentages ranged from 53% to 85% after 8 wk. An interaction between the auxin used and its concentration affected the mean number of roots produced ($P < 0.0001$). Rosettes formed similar numbers of roots among the NAA treatments (Table 4). The highest IBA concentration induced rosettes to form significantly more roots than rosettes on medium lacking IBA (controls). The presence of IAA in the media significantly decreased the number of roots formed. In the second experiment when rosettes remained on the IBA media for another 4 wk and IBA concentrations were higher, rooting percentages increased from 35% to 70% to 50% to 100% (Table 5). Also, more roots were produced by rosettes on the higher IBA concentrations.

At the higher auxin concentrations, more callus formed on the bases of the rosettes. Many of the roots appeared to have formed on callus rather than from the basal portion of the rosette. This callus with roots was easily knocked off when handling the rosettes.

Difficulties emerged in transferring rooted rosettes from tissue culture to *ex vitro* culture. After 2 mo, only 16% of the rosettes survived in pots that had been covered for 1 wk, while 37% of the rosettes survived in the uncovered pots. Covering the pots kept the soilless mix too moist and rosettes rotted. Flowers on surviving plants that bloomed appeared normal .

TABLE 4

Effects of different NAA, IBA, or IAA concentrations on in vitro *rooting of rosettes. Rooting percentages were based on 20 rosettes per treatment, and rosettes were from several plants. Roots were counted after 8 wk of treatment.*

Auxin	Concentration (µM)	Rooted (%)	Average root number per rosette [a]
NAA	0	65	6
	0.5	80	7
	1.0	70	6
	1.5	75	7
	2.0	81	7
IBA	0	65	6 bc
	0.5	65	4 d
	1.0	85	5 cd
	1.5	75	7 ab
	2.0	85	9 a
IAA	0	70	7 a
	0.5	55	2 c
	1.0	85	4 b
	1.5	70	4 b
	2.0	53	3 bc

[a] Comparisons of means are within a plant growth regulator treatment (N=20). For NAA, means were similar to each other ($P > 0.05$). For IBA and IAA, means with different letters are significantly different ($P < 0.05$).

TABLE 5

Effects of higher IBA concentrations and longer time in culture in in vitro *rooting of rosettes. Percentages based on 20 rosettes per treatment, and rosettes were from several plants. Roots were counted after 12 wk of treatment.*

IBA Concentration (µM)	Rooted (%) week 8	Rooted (%) week 12	Average root number per rosette [a]
0	35	50	4 d
2.5	70	95	8 c
4.9	70	95	10 b
7.4	70	95	10 b
9.8	70	100	16 a

[a] Means with different letters are significantly different ($P < 0.05$, N = 20)

SUMMARY OF MICROPROPAGATION PROTOCOL FOR *LEWISIA COTYLEDON*

1. Remove peduncle sections containing the lower axillary buds from flower inflorescences.

2. Surface disinfest peduncle sections by soaking them in a 10% bleach solution containing about 4 drops Tween 20 per liter (0.26 gal) for 20 min, then rinse 3 times in sterile distilled water.

3. Place peduncle sections in sterile vessels containing MS or DKW medium supplemented with 3.5 µM BA.

4. Place vessels under fluorescent lights at a constant 25 °C (77 °F) with a 16-h photoperiod.

5. Remove resulting rosettes, place on fresh medium (same as above) and allow to proliferate. Transfer rosettes to fresh medium monthly.

6. To root rosettes, place rosettes on MS medium supplemented with 2.5 µM IBA for 12 wk.

7. Transfer rooted rosettes to pots containing a soilless mix of 5 peat:4 perlite:3 coarse sand (by volume). Covering pots is unnecessary for rosette acclimation to natural environment. Water sparingly, allowing the top of the mix to dry out.

We established a micropropagation protocol for *Lewisia cotyledon*. The axillary buds on the lower part of the flower peduncle were used as the explant source for initiation of new rosettes in tissue culture. From observations in this study, rosette initiation from these axillary buds appeared to be independent of plant growth regulators or basal media tested. Genotype did have an effect as axillary buds removed from certain stock plants failed to initiate rosettes or those rosettes evidently died. Large numbers of rosettes were produced from a few initial rosettes within a year. For increasing and maintaining *in vitro* rosettes, DKW supplemented with 3.5 µM BA appeared to be a better basal medium for certain genotypes compared to MS or WPM. Tissue culture rosettes rooted *in vitro* after 12 wk on MS supplemented with up to 9.8 µM IBA. To keep callus proliferation to a minimum yet maximize root formation, a concentration of 2.5 µM IBA is recommended. *In vitro* rooted rosettes were successfully transferred to *ex vitro* culture and had normal phenotypes, but less than 40% of the transferred rosettes survived after 2 mo.

ACKNOWLEDGMENTS

Funding for these studies from the Perennial Plant Association is gratefully acknowledged. We also thank Rare Plant Research for providing the stock plants used for these experiments.

REFERENCES

Bailey LH, Bailey EZ. 1976. Hortus Third. New York (NY): Macmillan Publishing Co Inc.

Davidson BL. 2000. Lewisias. Portland (OR): Timber Press Inc. 236 p.

Lloyd G, McCown B. 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia,* by use of shoot-tip culture. Combined Proceedings of the International Plant Propagators Society 30:421–427.

McGranahan GH, Driver JA, Tulecke W. 1987. Tissue culture of Juglans. In: Bonga JM, Durzan DJ, editors. Cell and tissue culture in forestry, vol 3. Boston (MA): Martinus Nijhoff. p 261–271.

Mostul BL. 1995. Lewisia: a fast seller at the garden center. Digger 39(2):9, 15.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15:473–497.

SAS Institute Inc. 1999. SAS Online Doc®, version 8. Cary (NC): SAS Institute.

Sha L, McCown BH, Peterson LA. 1985. Occurrence and cause of shoot-tip necrosis in shoot cultures. Journal of the American Society for Horticultural Science 110:631–634.

USDA NRCS. 2002. The PLANTS database, version 3.5. URL:http://plants.usda.gov (accessed 20 Aug 2003). Baton Rouge (LA): The National Plants Data Center.

AUTHOR INFORMATION

Mary W George
Senior Scientific Aide
mgeorge@uidaho.edu

Robert R Tripepi
Professor
btripepi@uidaho.edu

Horticultural Sciences Division
Department of Plant, Soil
and Entomological Sciences
University of Idaho
Moscow, ID 83844-2339