Journal of Medicinally Active Plants

Volume 1 | Issue 1

January 2012

Effects of Cytokinin on Multiplication and Rooting of Aloe barbadensis during Micropropagation on Agar and Liquid Medium

Jeffrey Adelberg
Clemson University, jadlbrg@clemson.edu

Jacqueline Naylor-Adelberg
jnaylor@clemson.edu

Recommended Citation
Adelberg, Jeffrey, Jacqueline Naylor-Adelberg. 2012. "Effects of Cytokinin on Multiplication and Rooting of Aloe barbadensis during Micropropagation on Agar and Liquid Medium," *Journal of Medicinally Active Plants* 1(1):1-5.
DOI: https://doi.org/10.7275/R5251G4V
Available at: http://scholarworks.umass.edu/jmap/vol1/iss1/4

Effects of Cytokinin on Multiplication and Rooting of *Aloe barbadensis* during Micropropagation on Agar and Liquid Medium

Jeffrey Adelberg* and Jacqueline Naylor-Adelberg,

School of Agriculture, Forestry and Environmental Sciences, Clemson University, Clemson, SC 29634

***Corresponding author:** jadlbrg@clemson.edu

Manuscript received: July 27, 2011

Keywords: Acclimatization, benzyladenine, carryover effect, polyester matte, meta-topolin, xerophytes.

Abstract

Aloe barbadensis (syn. *Aloe vera*) was micropropagated on agar and liquid medium at varied benzyladenine (BA) and meta-topolin (MT) concentrations (0, 1, 3.2, and 10 µM) for three successive culture cycles and then transferred to a greenhouse for growth. MT induced multiplication at the highest concentration (10 µM) and BA produced the greatest number of plantlets (at 3.2 µM) with optimal multiplication at approximately 6 µM. Liquid medium did not affect multiplication rate when compared with agar, but plants were twice as large from liquid as compared with those from agar at the time of transfer to the greenhouse. After five weeks of growth, plants in the greenhouse micropropagated on liquid culture were still larger than plants micropropagated on agar with BA and MT. A carryover of cytokinin inhibited rooting, and plants on agar were more severely affected than plants on the liquid medium. Cytokinin carryover reduced rooting from 92% (control) to 68% with either the 3.2 µM MT or 10 µM BA and at 10 µM MT only about 20% of the aloe plants rooted. There appeared to be a trade-off between maximum multiplication rates and best plant quality for *ex vitro* transfer. Using liquid medium led to larger plants and lessened the cytokinin carryover effect on rooting without affecting the multiplication rate. Approximately 6 µM BA in liquid medium would be optimal for multiplication and rooting of *A. barbadensis*.

Introduction

Aloe barbadensis Mill. (syn. *Aloe vera* L.) is a xerophyte native to Africa and Arabia, but the exact origin is disputed. The aloe plant will not withstand a hard freeze and only grows in a climate similar to that of the Mediterranean area with mild wet winters and hot dry summers. While the plant can tolerate drought, no juice will be produced if the weather is too dry. Aloe flourishes in well-drained fertile soil and requires about 1 m^2 of field space for growth and development.

While the demand for aloe is increasing, cultivation is slow as seed does not often form on plants in cultivation, and when available, these seeds can take years to germinate. Propagation is typically done from offsets, although these take two to three years of field growth to reach a harvestable size. Vegetative propagation is generally too slow for cultivation purposes. Successful micropropagation of aloe would minimize the large requirements for time and space in field propagation and has been proposed to meet the need for large numbers of plants (Ken Altman, personal communication).

In commercial horticulture, micropropagation usually involves enhanced axillary divisions and is most often induced by the synthetic cytokinin, benzyladenine (BA). BA retained in plants during subsequent transfers, known as cytokinin-carryover, lowers the rooting response in the greenhouse. For example, BA accumulated in the basal portion of

Spathiphyllum floribundum and inhibited rooting *in vitro* and *ex vitro* (Werbrouk *et al.,* 1995).

Meta-topolin (MT), a BA analog readily degrades during acclimatization so rooting can be combined with axillary shoot division (Werbrouk *et al.,* 1995). In *Aloe polyphylla*, BA interfered with rooting and caused hyperhydricity, resulting in plant loss upon transfer to a greenhouse, whereas MT allowed successful *in vitro* rooting and eliminated hyperhydricity (Bariu *et al.,* 2007). In liquid micropropagation systems for plantain, 4.4 µM MT was optimal among all the cytokinins tested (Escalona *et al.,* 2003; Roels *et al.,* 2005). MT proved particularly useful in enabling rooting of difficult genotypes in potato and sea oats when concentration was balanced for each genotype (Baroja-Fernandez *et al.,* 2002; Valero-Arcama *et al.,* 2010). In turmeric (*Cucurma longa* L.), however, MT caused higher rates of shoot multiplication than BA, but the roots were short and thin (Salvi *et al.,* 2002).

Our laboratory has had prior successes in micropropagation on liquid medium as an alternative to agar (Adelberg, 2004; Adelberg and Fari, 2010). Generally, plants propagated on liquid media multiply more rapidly, are larger in size, and grow more quickly in the greenhouse. Yet, hyperhydricity can occur when plants are immersed in liquid medium.

In preliminary work that included twelve species of xerophytes in the genera *Aloe*, *Agave*, and *Echeveria,* hyperhydricity was a problem when tissues were immersed in a liquid medium (unpublished data). A liquid infused, polyester fiber matte developed in our laboratory reduced hyperhydricity in liquid culture without mechanical aeration (Tascan *et al.,* 2010). The objectives of this research were to test micropropagation of *A. barbadensis* in an agar and liquid matte system, using selected concentrations of BA and MT, for multiplication, rooting, and greenhouse growth.

Materials and Methods

Explant preparation.

Aloe barbadensis, purchased from Altman Plants (Vista, CA), was used in this study. The plants were surface drenched with 1 µM benzyladenine (BA) to encourage offsets. After the offsets developed, they were collected and cleaned in Tween 20 detergent water while being constantly stirred in a beaker. After cleaning, the plant tissue was rinsed in distilled water and the larger leaves were removed and placed in a 90% ethanol dip for 1- 2 min and then 1-2 min in 5.25% NaClO (100% Clorox, Oakland, CA), and rinsed with sterile, deionized water within a laminar flow hood to prevent contamination. Damaged tissue was removed and the shoot tips were placed in test tubes of Murashige and Skoog (1962) medium, 1 µM BA, 30 g L^{-1} sucrose, 7 g L^{-1} agar, supplemented with 2 mg L^{-1} glycine, 100 mg L^{-1} myo-inositol, 0.5 mg L^{-1} nicotinic acid, 0.5 mg L^{-1} pyridoxine·HCL, 0.1 mg L^{-1} thiamine·HCl. Stage II explants were cultured on the same medium with 3 µM BA, 5% sucrose with 33 mL of medium in 180 mL glass jars for five weeks with 25 to 35 µmol s^{-1} m^{-2} PAR provided by cool white fluorescent tubes with 16 h d^{-1} photoperiod maintained at 24 $\pm$ 2°C.

Effects of cytokinin and physical environment

The effects of cytokinin and media structure were tested using an agar and liquid medium amended with BA or MT at 0, 1, 3.2,10 µM and arranged in factorial combinations. Both the agar media (40 mL per vessel) and liquid media (35 mL per vessel) were contained in Magenta GA7 boxes (Magenta Corp., Chicago, IL, USA). The base structure of the liquid media was formed using a 5 cm x 5 cm square Anchor Heavy Weight Seed Germination Paper (Anchor Paper Co., St Paul, MN, USA) supported on a 5 cm x 5 cm low-loft polyester matte (Fairfield Processing, Danbury, CT).

A total of three stage II explants were placed in each vessel and cultured for five weeks. The entire experiment was repeated three times sequentially with plant multiplication recorded following each repeat. At the end of the third cycle all plants were moved to soilless mix Fafard 3-B (Canadian sphagnum peat moss, 3/8" Processed pine bark, perlite, vermiculite, wetting agent, and dolomitic limestone; Fafard Co., Anderson, SC). After five weeks in greenhouse conditions (minimum temperatures of 25°C day and 15°C night, latitude of 34.67350, longitude = 82.83261, 60% shade

cloth; misting cycle of 6 second every 16 min during daylight hours). Survival and growth of the cultured plants were recorded at the end of the five weeks and an analysis of Variance (ANOVA) was used to determine any significant differences in plant growth. Regression and means separation significant at Prob. > F 0.05 were presented.

Results and Discussion

Multiplication of *A. barbadensis* is slow in the laboratory, similar to reproduction in the field. In the absence of exogenous cytokinins, very few off-sets were formed and little or no multiplication occurred in the five-week culture periods (1.0x – 1.4x, 95% confidence interval). Meta-topolin was effective in promoting plant multiplication at a concentration of 10 µM, the highest concentration tested. BA promoted multiplication of plants in the 1 – 10 µM range and at the optimal concentration of 6 µM, BA would more than double the number of plants in the five week growth cycle (Figure 1). The multiplication response was greater with BA than MT in the 1-10 µM range.

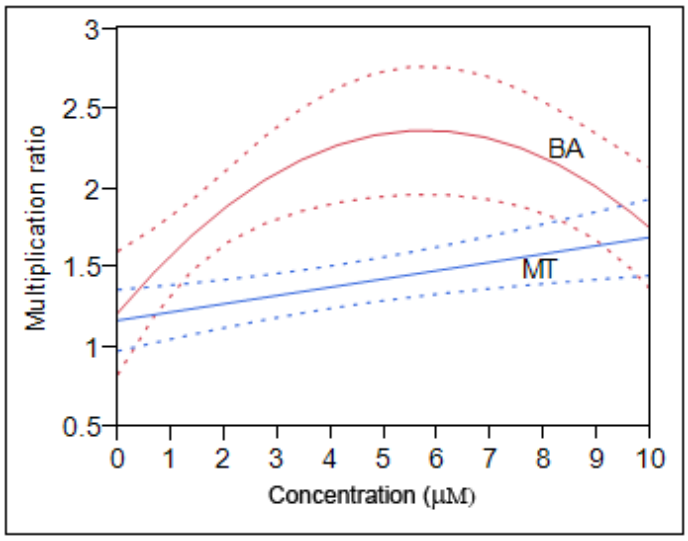

Figure 1. Multiplication of *A. barbadensis* plants in agar and liquid with concentrations of BA and MT.
Multiplication ratios (number of plants formed per original plant) = 1.75 + 0.12 (µM BA) + 0.04 (µM BA)^2; R^2 = 0.13; and. 1.17 + 0.05(µM MT); R^2 = 0.11. Dashed lines indicate 95% confidence limits.

Plants grown in the laboratory on liquid medium appeared larger than those grown on agar medium (Figure 2). This was quantified during the last growth cycle, when plant mass was recorded immediately prior to transfer to the greenhouse. The plants grown on agar medium averaged 1.34 ± 0.33 g per plant, and the plants grown on liquid averaged 2.79 ± 0.20 g per plant (Figure 2). When grown on the mist bed, the larger plants did not shrink or wither to any noticeable extent. Hyperhydricity was not observed in any of the liquid-matte cultures plants or on any of the plants grown on BA, although both liquid and BA had been previously associated with hyperhydricity with other plants in our laboratory. Many of the plantlets had rooted *in vitro* and an increase in cytokinin reduced the numbers of *in vitro* roots (data not shown).

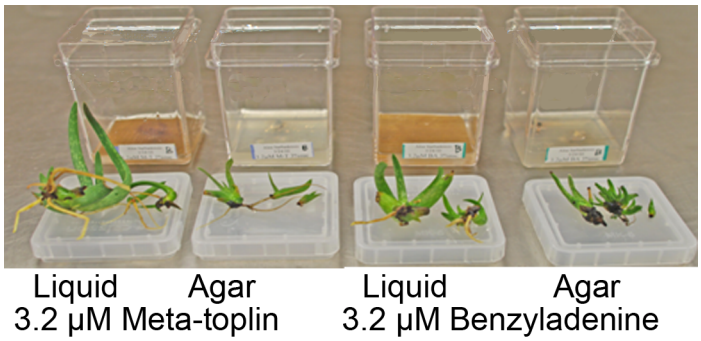

Figure 2. Plantlets after five weeks on liquid and agar medium with indicated cytokinin.
Plants on liquid medium averaged 2.79 g per plant.
Plants on agar medium averaged 1.34 g per plant.

Rooting of plantlets in a soilless mix in the greenhouse is a critical response for survival *ex vitro*. After five weeks in the greenhouse, 92% of the plantlets from media without cytokinins had rooted. Cytokinin carryover effects, perhaps from residual BA in the plant tissue, reduced the numbers of plants that had rooted (Figure 3). The reduction in rooting was greater on agar, than on liquid medium, and the greatest reductions in rooting occurred with the highest concentrations of cytokinins in agar medium. The significant 3rd order interactions (PGR x concentration x media type) was caused by mortality in agar-produced plants at the highest concentration of meta-topolin. Responses for either 3.2 µM or10 µM BA in either media gave adequate rooting (about 68%). The

larger plants from liquid culture appeared less prone to the cytokinin carryover due to either increased vigor or some other diminution of the assumed cytokinin residual.

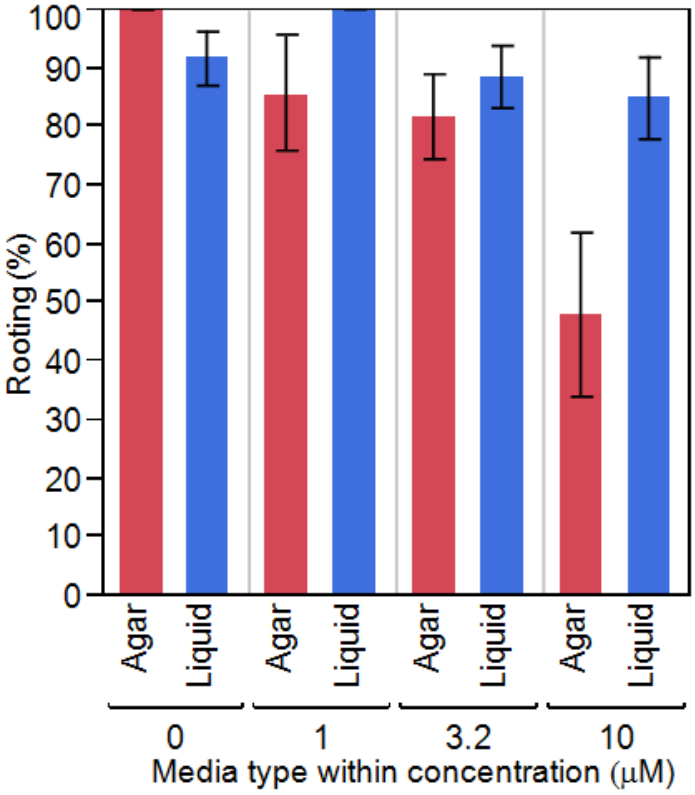

Figure 3. Rooting of plants after five weeks growth.
Means of BA and MT treatments; reduced rooting as compared with control (no cytokinin) demonstrates carry-over effect.

During acclimatization, tissue cultured plants are known to vary in size. Quality-oriented commercial nurseries sort young plants to capture value from the larger-sized variants. MT reduced plant size in agar and BA reduced plant size on both agar and liquid (Table 1). The larger plants from liquid culture remained larger after five weeks growth in the greenhouse (Figure 4). Larger plants from the laboratory retained their size advantage in the nursery. This confers an economic advantage as for many commercial propagators larger plants command a higher sale price.

In this study with *A. barbadensis*, MT was less effective than BA for multiplication and did not improve the rooting percentage when compared to BA. Producing *A. barbadensis* on BA (6 µM) would result in the greatest number of plantlets with adequate rooting and moderate size. Concentrations of MT greater than 10 µM may be tested to achieve higher rates of axillary division, but reduced rooting due to cytokinin carryover would likely be problematic after transfer to the greenhouse for growth and development of the plants. Although both cytokinins reduced plant size due and cytokinin carryover, the effect on the liquid medium plants was smaller than for the agar medium plants. Apparently, the larger plantlets from the liquid medium counteracted some of the size reductions from cytokinin use. Therefore, liquid medium with 6 µM BA is indicated as optimal for culture of *A. barbadensis*.

Our results support the conclusions of Werbrouk (2010) on his review of aromatic cytokinins for tissue culture. New compounds, such as MT, may further expand our choices of cytokinin, but probably none will be the perfect substance for every plant tissue culture problem (Werbrouk, 2010).

Table 1. Changes in plant size caused by BA and MT.

Cytokinin concentration	BA		MT	
	Agar	Liquid	Agar	Liquid
(µM)	(Longest leaf (mm)[1]		(Longest leaf (mm)[1]	
0	94	85	94	85
1	30	64	55	64
3.2	39	52	51	82
10	26	43	0	105

[1]Measured after five weeks growth in greenhouse.

Figure 4. Plant growth after transfer to greenhouse.

Larger plants were multiplied in liquid media.

Acknowledgment: The author's would like to thank Altman Plants, Vista CA, for their generous support of this project.

References

Adelberg, J. 2004. Plant growth and sugar utilization in an agitated, thin film liquid system for micropropagation. In Vitro Cell. Dev. Biol.—Plant 40:245-250.

Adelberg, J., and M. Fari. 2010. Applied physiology and practical bioreactor designs for micropropagation of ornamental plants. Propagation of Ornamental Plants 10:205-219.

Bairu M.W., W.A.Stirk, K. Dolezal, and J.Van Staden. 2007. Optimizing the micropropagation protocol for the endangered *Aloe polyphylla*: can meta-topolin and its derivatives serve as a replacement for benzyladenine and zeatin? Plant Cell Tiss Org. 90:15-23.

Baroja-Fernandez, E., J. Aguirreola, H. Martinkova, J. Hanus, and M. Strnad. 2002. Aromatic cytokinins in micrpropagated potato plants. Plant Physiol. Biochem. 40:217- 227.

Escalona, M., I. Cejas, J. Gonzales-Olmedo, I. Capote, S. Roels, M.J. Canal, R. Rodriguez, J. Sandolval, and P. Debergh. 2003. The effects of meta-topolin on plantain propagation using a temporary immersion bioreactor. Infomusa 12:28-30.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Roels, S., M. Escalona, I. Cejas, C. Noceda, R. Rodriguez, M.J. Canal, J. Sandolval, and P. Debergh. 2005. Optimization of plantain (Musa AAB) micropropagation by temporary immersion system. Plant Cell Tiss Org. 82:57-66.

Salvi, N.D., L. George, and S. Eapen. 2002. Micropropagation and field evaluation of micropropagated plants of turmeric. Plant Cell Tiss Org. 68:143–151.

Tascan, A., J. Adelberg, M. Tascan, N. Joshee, and A. Yadav. 2010. Polyester fiber controlled hyperhydricity of *Scutellaria* species in *in vitro* liquid culture systems. HortScience 45:1723-1728.

Valero-Arcama, C., M.E. Kane, S.B.Wilson, and N.P. Philman. 2010. Substitution of benzyladenine with meta-topolin during shoot multiplication increases acclimatization of difficult- and easy-to acclimatize sea oats (*Uniola paniculata* L.) genotypes. Plant Growth Regul. 60:43-49.

Werbrouk, S.P.O., B. van der Jeugt, W. Dewitte, E. Prinsen, H.A. van Onckelen, and P.C. Debebergh. 1995. The metabolism of benzyladenine in *Spathiphyllum floribundum* Schott. 'Petite' in relation to acclimatization problems. Plant Cell Rep. 14:662-665.

Werbrouk, S.P.O. 2010. Merits and drawbacks of new aromatic cytokinins in plant tissue culture. Acta. Hort. 865:103-107.