Establishment and multiplication of firechalice in plant tissue culture©

A.A. Alosaimi and R.R. Tripepi[a]

Plant Science Division, PSES, University of Idaho, Moscow, Idaho 83844-2339, USA.

Abstract

Firechalice, *Epilobium canum* (Greene) P.H. Raven subsp. *garrettii* (A. Nelson) P.H. Raven, is a small and thinly branched plant that is difficult to germinate from seed. In order to increase the number of selected individuals rapidly, plant tissue culture would be the propagation method of choice. Single-node stem explants from a selected plant were examined for their ability to establish on Murashige and Skoog (MS) medium or Woody Plant Medium (WPM). Murashige and Skoog medium was found the best salt formulation particularly when supplemented with 4.4 µM benzyladenine (BA). During Stage 2, different plant growth regulators, such as BA, kinetin (Kin), 6-(γ,γ-dimethlyallylamino) purine (2iP), thidiazuron (TDZ) and meta-topolin (mT), were used in the media in different concentrations (1.1, 2.2, 4.4 or 8.8 µM). All the cytokinins tested induced the explants to form the most shoots and shoot dry weight when used at 4.4 or 8.8 µM in the medium. A concentration of 8.8 µM BA or mT were most effective for promoting shoot multiplication, with these concentrations inducing means of 13.7 or 14.1 shoots per explant, respectively. All but one cytokinin failed to affect shoot heights at the highest concentrations used, but 4.4 or 8.8 µM TDZ decreased shoot height by at least 54% compared to the control shoots. These results indicated that firechalice shoots established the best on MS medium for Stage 1 and 4.4 or 8.8 µM meta-topolin in the medium resulted in explants forming the most and largest shoots during Stage 2.

INTRODUCTION

Epilobium canum subsp. *garrettii* (also known as *Zauschneria garrettii*) common name firechalice or hummingbird flower is in *Onagraceae* family. This species is sometimes called "orange carpet" because the plant spreads as a ground cover, and its flowers are bright orange-red and attractive to hummingbirds. This species is relatively small, usually 30 to 46 cm tall and 30 to 61 cm wide (Love et al., 2009). Since plants grow easily in dry areas and have several good characteristics that are useful for urban landscapes, the plants should be propagated asexually to retain the desired characteristics. Axillary shoot proliferation is the best tissue culture technique for true-to-type reproduction. Plants used in axillary shoot culture will undergo the four stages of micropropagation. Stage 1 is establishment and stabilization of shoot cultures. During this stage the best basal medium to use must be determined. For example, Murashige and Skoog (MS) medium, Woody Plant Medium (WPM), or Driver-Kuniyuki walnut (DKW) medium can be used to establish shoots in vitro. Stage 2 involves inducing axillary shoot proliferation by increasing the level of cytokinin in the medium. Explants usually respond to high concentrations of cytokinin and produce many shoots (Einset, 1986). Benzyladenine is the most widely used cytokinin in the micropropagation industry, yet meta-topolin a relatively new synthetic cytokinins, can be used as an alternative to BA and zeatin.

RESEARCH OBJECTIVE

The goal of this research was to develop a micropropagation procedure for rapid production of a selected firechalice plant that was collected near Tony Grove Lake, Cache County, in northern Utah. We demonstrate that firechalice can multiply quickly in the first

[a]E-mail: btripepi@uidaho.edu

two stages of micropropagation so that hundreds or thousands of a selected clone can be made available to production nurseries.

MATERIAL AND METHODS

Stage 1

Firechalice shoots were established in tissue culture by testing two types of media. Single-node explants were placed on MS medium (Murashige and Skoog 1962) or WPM (Lloyd and McCown 1980). Murashige and Skoog medium contained 4.3 g L^{-1} mineral salts and 5.9 µM thiamine-HCl, 8.1 µM nicotinic acid, 4.9 µM pyridoxine-HCl, 53.3 µM glycine, 100 mg L^{-1} myo-inositol, 30 g L^{-1} sucrose, solidified with 7 g L^{-1} agar, was adjusted to pH 5.7, and included 4.4 µM BA. Woody Plant Medium contained 2.3 g L^{-1} salts, the same concentrations of thiamine, nicotinic acid, pyridoxine, glycine, and myo-inositol as MS medium, and contained 20 g L^{-1} sucrose, was solidified with 7 g L^{-1} agar, was adjusted to pH 5.2 and included 3.5 µM BA.

Stage 2

Shoot explants used in this part of the study were taken from shoot cultures grown on MS medium supplemented with 4.4 µM BA. Different cytokinins in different concentrations were used: benzylaminopurine (BA), Kinetin (Kin), 6-(γ,γ-dimethyl allylamino)-purine (2iP), Thidiazuron (TDZ), or meta-topolin (mT) at 0, 1.1, 2.2, 4.4, or 8.8 µM. Stem explants ~1 cm tall were placed on MS media containing different cytokinins and grown for 30 days before taking data. Statistical analyses for number of shoots, shoot height, and shoot dry weight were analyzed as by two-way analysis of variance (mixed model procedure) (Proc Mixed, SAS 2012) when comparing different plant growth regulators used at various concentrations. For Stage 2 analyses, cytokinin and cytokinin concentrations were used as independent variables. If the interaction between the cytokinins and their concentrations was significant for a growth parameter, then effects of the growth regulator concentrations were tested for each individual growth regulator. Significant differences between treatment means were determined by least-square means at the 5% level when comparing plant growth differences of explants placed on different media.

RESULTS

In Stage 1, shoot explants on MS medium produced at least 2 fold more new shoots, grew almost 3 times taller, and produced 4 fold more shoot dry weight than those on WPM (data not shown). After three subcultures of firechalice shoots on MS medium containing 4.4 µM BA, the shoots had stable growth (consistent foliage size and color), and the shoots were then used in Stage 2 experiments.

The effects of cytokinins in Stage 2 had to be analyzed separately due to an interaction between type of cytokinin and cytokinin concentrations. The two most effective cytokinins for promoting shoot multiplication were BA and mT. A concentration of 8.8 µM BA induced about 13.7 shoots to form per explant, whereas 4.4 µM mT induced 13.5 shoots to form per explant (Table 1 and Figure 1). The highest BA concentration (8.8 µM) increased shoot dry weight ~2.3 fold compared to the control stems. In contrast, 8.8 µM meta-topolin increased shoot dry weight about 2.6 fold compared to control shoots.

The other three cytokinins used in this study either had minimal or detrimental effects on the growth of firechalice shoots. For instance, even though 8.8 µM TDZ increased the number of axillary shoots formed by 2.9 fold and shoot dry weight by 4 fold over the control treatment, shoots height on medium supplemented with 8.8 µM TDZ were 2.5 times shorter than control shoots. Neither Kin nor 2iP concentrations affected shoot heights, yet 8.8 µM kin or 2iP increased shoot dry weights by 2.3 fold each compared to controls.

Table 1. Effects of plant growth regulators (cytokinins) on the mean number of shoots, mean shoot heights, and mean shoot dry weights of firechalice shoots grown on MS medium for 4 weeks. Data are means of six shoots in four vessels per treatment.

Plant growth regulator	Concentration (µM)	Number of shoots	Shoot height (cm)	Shoot dry weight (mg)
BA	0	3.1 a[1]	3	25 a
	1.1	7.4 b	3	32 ab
	2.2	10.6 bc	3.7	34 ab
	4.4	10.2 bc	2.9	36 b
	8.8	13.7 c	2.6	57 c
mT	0	2.3 a	3	27 a
	1.1	7.1 b	3.8	35 ab
	2.2	8.5 b	3.1	60 bc
	4.4	13.5 c	3.7	65 bc
	8.8	14.1 c	3.3	72 c
TDZ	0	2.1 a	3.5 d	24 a
	1.1	5.5 b	2.4 c	36 a
	2.2	5.2 b	2.1 bc	41 a
	4.4	5 b	1.6 ab	71 ab
	8.8	6.2 b	1.4 a	98 b
Kin	0	1.9 a	2.4	16 a
	1.1	3.1 b	3.0	25 ab
	2.2	3.9 b	2.9	32 b
	4.4	3.9 b	2.7	31 b
	8.8	7.9 c	3.3	36 b
2iP	0	2.5 a	3.0	23 a
	1.1	3.3 ab	2.7	19 a
	2.2	3.8 b	2.8	33 ab
	4.4	4.3 b	2.5	34 ab
	8.8	6.2 c	3.1	44 b

[1]Different letters within a column for each individual growth regulator indicate significant differences between means as determined by least-squares means tests at $P \leq 0.05$ level (n=24).

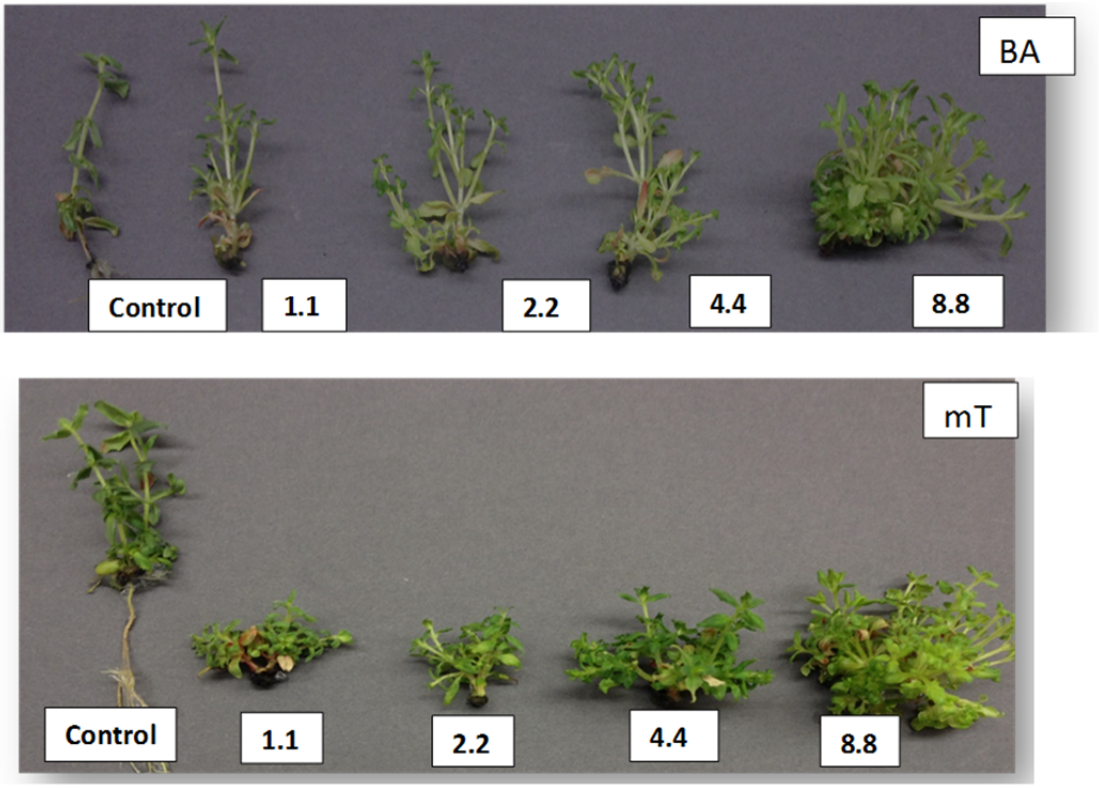

Figure 1. Effects of different concentrations of BA and mT on firechalice shoot multiplication after shoots were grown on MS medium for 4 weeks.

DISCUSSION

In Stage 2 studies with firechalice, mT promoted shoot multiplication the best, even a little better than BA. This information is important for propagators who have to decide which cytokinins to use in their media. Besides looking for the best plant responses in culture, propagators must also consider the costs of the biochemicals used. The cost of mT from *Phyto*Technology Laboratories in 2015 was $257 per gram, whereas the cost of BA from this same company was $5 per gram. The higher cost of mT failed to justify its use in commercial propagation since BA, which was 51 times cheaper, promoted shoot multiplication almost as well as mT. In contrast, addition of TDZ to shoot multiplication medium should be avoided since it inhibited shoot height growth of firechalice.

CONCLUSION

Exact duplicate plants could be rapidly increased for firechalice by using in vitro culture. MS medium was the best medium for establishing firechalice stem explants in Stage 1. During Stage 2, shoot explants were multiplied the best by using BA or mT at 4.4 or 8.8 µM.

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