HortScience 24(3):503–504. 1989.

Shoot Proliferation of *Euphorbia fulgens* in Vitro Affected by Medium Components

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Additional index words. tissue culture, in vitro propagation, zeatin, sucrose, medium pH

Abstract. **Effects of *trans*-zeatin, sucrose, *myo*-inositol, and medium pH on shoot proliferation of *Euphorbia fulgens* Karw. ex Klostch were studied in vitro. Maximum shoot production occurred on media supplemented with 5 μM zeatin, but maximum shoot length with 5 to 15 μM. Shoot production increased with sucrose concentration, and was maximal at 131.5 mM. *myo*-Inositol concentration up to 0.6 mM did not have a significant effect on shoot production, but >1.1 mM reduced it. The optimal medium pH was 5.3 for shoot proliferation, but lower pH values stimulated shoot growth. Chemical names used: *trans*-2-methyl-4-(1*H*-purin-6-ylamino)-2-buten-1-ol (*trans*-zeatin), α-D-glucopyranosyl-β-D-fructofuranoside (sucrose).**

Euphorbia fulgens (also called Scarlet Plume), a medium-sized shrub from Mexico, is generally propagated by cuttage and grown as a cut flower in greenhouses for cutting from January to March in temperate zones (Post, 1952). There are several cultivars with flower colors from white to orange and red. In vitro propagation of *E. fulgens* has proved to be faster and requires less greenhouse space than conventional cuttage (Zhang et al., 1987). Zeatin, though expensive, is required for shoot initiation and multiplication of *E. fulgens* in vitro (Zhang et al., 1987). In our previous study, high zeatin levels resulted in increased bud initiation, but they also inhibited shoot growth. Maximum shoot production occurred when explants were transferred at 4-week intervals from 4.5 to 23.0 to 4.5 μM zeatin-enriched media. The present study was initiated to determine if shoot production might still be enhanced by providing relatively low levels of zeatin and proper subculturing. In addition, we wanted to study the effects of levels of *myo*-inositol, sucrose, and medium pH on shoot production of *E. fulgens* in vitro.

Plant materials. Plants of *Euphorbia fulgens* (the red cultivar), grown in pots on an open greenhouse bench for 1 year, were used as the explant source. The plants were watered and fertilized as needed. To enhance sterilization efficiency, no overhead watering was applied during the time when explants were being selected. Actively growing apical shoots, ≈10 cm long with five to six nodes each, were selected. After excision, the shoots were surface-sterilized as described by Zhang et al. (1987). The apical shoots were then cut to provide single-node sections 10 mm long, each with one lateral bud.

Culture conditions. Except when indicated, the basal nutrient medium for all cultures contained inorganic salts of Murashige and Skoog (MS) (1962) revised medium, with the exception of Fe which was used as formulated by de Fossard (1976), 4 μM nicotinic acid, 2.4 μM pyridoxine·HCl, 0.3 μM thiamine·HCl, 26.6 μM glycine, 0.56 mM *myo*-inositol, 73.0 mM sucrose, and 8 g Difco Bacto-agar/liter. Unless otherwise stated, all media were adjusted to pH 5.8 before autoclaving at 121C and 110 kPa for 20 min.

Culture tubes (25 × 100 mm), each with 10 ml of medium, and glass jars (50 mm in diameter × 105 mm high), each containing 25 ml of medium, were used. Culture tubes were used for explant cultures, glass jars for subcultures transferred from the tubes. The culture room was maintained at 25 ± 1C with a 16-hr daily light period supplied by cool-white fluorescent lamps that provided 50 $\mu mol \cdot s^{-1} \cdot m^{-2}$ at the top of culture containers.

Shoot proliferation. Effects of selected medium components on microshoot production were studied using nodal sections from the greenhouse-grown plants described previously. For the first 4 weeks, the nodal sections were placed in tubes containing basal medium with one component varied for shoot initiation. Component concentrations in the first 4-week culture period were: a) *trans*-zeatin, 0, 2.5, 5, 10, 15, 25, 30, or 40 μM; b) sucrose, 14.6, 43.8, 73.0, 102.3, 131.5, 160.7, or 189.9 mM; c) *myo*-inositol, 0, 0.28, 0.56, or 1.1 mM. Medium pH was 4.8, 5.3,

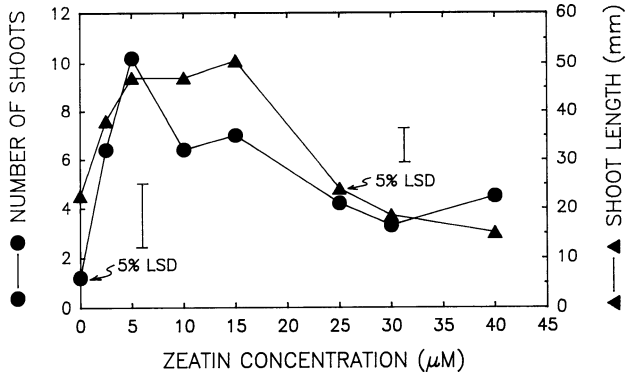

Fig. 1. Effect of *trans*-zeatin concentration in MS medium during the first 4-week culture on *E. fulgens* (red cultivar) mean shoot number and length. Data were taken after an additional 4-week culture at 2.5 μM zeatin, n = 20.

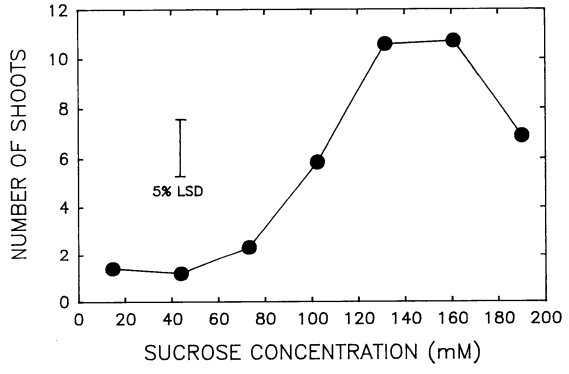

Fig. 2. Effect of sucrose concentration on shoot number of *E. fulgens* (red cultivar) growing on MS medium supplemented with 9.1 μM zeatin for the first 4-week culture and 2.5 μM for the second 4-week culture, n = 20.

Received for publication 8 Sept. 1987. The investigation reported in this paper (87-10-131) is in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the Director. We are grateful to Douglas D. Archbold and Robert G. Anderson for their careful review and corrections. The plant materials used in this study were obtained from Faber Greenhouses, Kankakee, IL 60901. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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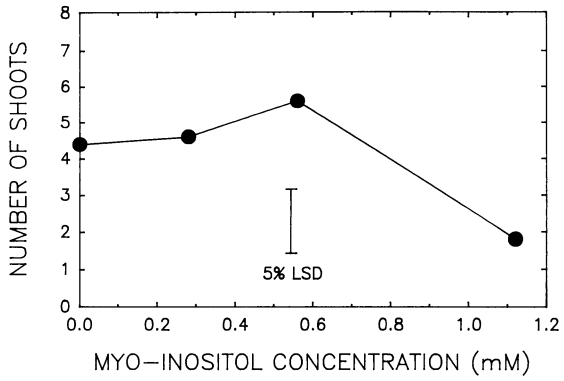

Fig. 3. Effect of *myo*-inositol concentration on shoot number of *E. fulgens* (red cultivar) growing on MS medium supplemented with 9.1 μM zeatin for the first 4-week culture and 2.5 μM for the second 4-week culture, n = 20.

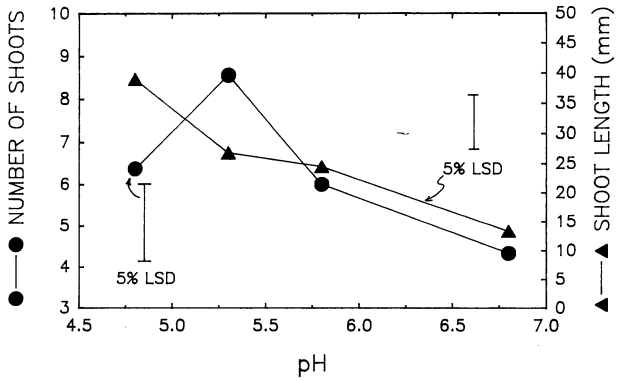

Fig. 4. Effect of pH on shoot number and length of *E. fulgens* (red cultivar) growing on MS medium supplemented with 9.1 μM zeatin for the first 4-week culture and 2.5 μM for the second 4-week culture, n = 20.

5.8, 6.3, or 6.8. For the second 4-week culture period, the original explants were transferred to glass jars all containing the same medium, consisting of basal medium and 2.5 μM zeatin to stimulate the formation of rootable microshoots (>5 mm).

Zeatin level. Among zeatin levels tested, 5 μM was optimum for shoot production (Fig. 1). The medium without zeatin yielded only one shoot per nodal explant. Shoot growth was less sensitive to zeatin level than shoot number; the best range of zeatin levels for shoot growth was 5 to 15 μM, a wider range than optimal for shoot production. High levels of zeatin (>15 μM) inhibited both shoot production and shoot growth (Fig. 1). Our previous work showed that two 4-week culture periods for the explants on medium supplemented with 9.1 μM zeatin resulted in 4.3 shoots per culture; this level of zeatin inhibited the adventitious buds from developing into shoots during the second 4-week culture period (Zhang et al., 1987). The present study shows that two 4-week culture periods on media with different zeatin levels (5 μM followed by 2.5 μM) gave rise to ≈10 shoots per culture. Almost all adventitious buds initiated on 5 μM zeatin medium during first 4-week culture period developed into shoots ≥5 mm on the 2.5-μM zeatin medium during the second 4-week culture period.

Sucrose concentration. Shoot number per culture was not significantly affected by sucrose at 14.6 to 73.0 μM, but increased significantly at 73.0 to 131.5 mM. Shoot production did not change or decrease significantly at higher concentrations (Fig. 2). The best level of sucrose for shoot production was between ≈130 and 160 mM, which is much higher than that (73.0 mM) usually employed (Murashige, 1974). High sucrose levels have been reported to increase shoot proliferation of chrysanthemum in vitro (Roest and Bockelmann, 1975).

myo-*Inositol concentration*. Shoot production at 0.56 mM *myo*-inositol was highest, but not significantly different from that at 0 and 0.28 mM (Fig. 3). *myo*-Inositol has been reported not to be essential for many plant species, but its presence at 0.56 mM, as in the present study, has been reported to be beneficial for shoot proliferation in vitro (Murashige, 1974). However, doubling the recommended level significantly reduced shoot production.

Medium pH. Both shoot production and length were sensitive to medium pH. For shoot production, pH 5.3 was optimal (Fig. 4). Shoot length decreased as pH increased above pH 4.8. These pH values are lower than that used (5.8) for many plants (Murashige, 1974). However, low pH values have been reported to be optimal for in vitro shoot proliferation and growth of rhododendrons (Anderson, 1975). Actual medium pH values after autoclaving and during culture may have changed (George and Sherrington, 1984). Skirvin et al. (1986) reported that the actual post-autoclaving medium pH values decreased due to the addition of plant tissues.

Our previous work reported maximum shoot production of *E. fulgens* using MS medium supplemented with 9.1 μM zeatin when sucrose was held constant at 73.0 mM and pH adjusted to 5.8 before autoclaving (Zhang et al., 1987). In the present study, maximum number of shoots was produced either by increasing sucrose to 130 to 160 mM, reducing medium pH to 5.3 before autoclaving, or supplementing the medium with 5 μM zeatin. This study demonstrates that zeatin levels as low as 5 μM can induce the nodal explants of *E. fulgens* to initiate and form many rootable shoots. The in vitro propagation of *E. fulgens* should be commercially applicable by employing optimal levels of zeatin, sucrose, *myo*-inositol, and pH, as well as the subculturing protocols used in this study. A comparison of shoot production from nodal explants of the red, white, and orange cultivars showed similar responses among all three cultivars.

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