Efficient Propagation Technique of *Euphorbia × lomi* Thai Hybrids

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Abstract. A simple and efficient in vitro propagation protocol of two cultivars of *Euphorbia × lomi* Rauh is described. Mother plants of ‘Nguen Muang’ and ‘Porn Ying Yai’ were soilless grown in 1 coconut coir dust:1 perlite (by volume) and 2 sphagnum peat:1 perlite (by volume) to produce cuttings. Plants cultured in peat-based substrate showed thicker stems (36.0 versus 30.5 mm, respectively), higher production of cuttings (10.4 versus 5.6 per plant), higher water absorption (265.7 versus 153.5 mL/plan/d), and higher content of nutrients in the root zone as compared with those cultivated in coir dust mixture. Cuttings harvested from 1-year-old mother plants of both cultivars were treated with 4000 ppm of α-naphthalene acetic acid (NAA) powder and inserted into bottom-heated benches filled with different substrates [perlite, 1 perlite:1 coir dust (by volume), coir dust] for rooting. Cuttings in pure perlite evidenced higher rooting percentage (86.2%) and longer roots (6.2 cm) than those in other media. Application of NAA resulted in higher rooting capacity (78.8 versus 68.3%, respectively) and higher amount of roots per cutting (13.4 versus 10.2) than treatment without auxin. Rooted cuttings grown in 1 peat:1 perlite medium (by volume) produced higher marketable potted plants than in 2 peat:1 perlite medium.

*Euphorbia × lomi* Rauh, an interspecific hybrid of *Euphorbia milii* Des Moulins (or Crown-of-thorns) and *E. lophogona* Lamarck, both endemic to Madagascar (Graf, 1985), was recently introduced to the Mediterranean countries as a new floral crop according to the increased demand for new low-maintenance ornamental plants. *Euphorbia × lomi* is a succulent shrub characterized by an erect, thick, fleshy, branched spiny stems up to 1 to 1.5 m long with long, lanceolate leaves and brightly colored bracts (Rauh, 1979). These hybrids can be year-round grown in dry, high temperature and high solar radiation areas as potted, bedding, or garden plants (Jankalski, 2000).

Crown-of-thorns and the hybrids are generally propagated by tip cuttings (Smoley, 2000), which is the most economic way to produce these plants, but information on the vegetative propagation is still limited. Mother plants are normally cultivated in soil, so disease problems such as root rot are frequent if drainage is not adequate and there is no possibility to monitor plant uptake with dispersion of water and nutrient excesses. V-cleft grafting could reduce the risk of rot associated with direct rooted cuttings but limit large-scale propagation (McLaughlin and Garofalo, 2002). Seeds should not be used to propagate these genotypes because of producing extremely variable seedlings. Recently, *Euphorbia milii* species was also introduced and cultured in vitro (Airo` et al., 2007), also by using a bioreactor system (Dewir et al., 2005). However, tissue culture is very expensive and often requires a high amount of labor (Saranga and Cameron, 2007).

Therefore, in 2004, a research program was initiated at the Research Unit for Mediterranean Flower Species of Palermo, Italy, to develop a comprehensive vegetative propagation system. The objective of this study involved the following steps: 1) growing mother plants of two *Euphorbia × lomi* Thai hybrids year-round in soilless media; 2) rooting cuttings harvested from 1-year-old mother plants in different media with or without auxin application; and 3) planting rooted cuttings to produce potted plants. The experiments were conducted on two cultivars: 1) to evaluate the effect of soilless media on plant growth and cuttings production; 2) to determine the influence of substrates and auxin application on rooting; and 3) to study the effect of growing media on growth of rooted cuttings and pot plant production.

Materials and Methods

Expt. 1: Mother plant cultivation. This trial was carried out in a double-span polyethylene (PE) -covered 540 m² greenhouse (28 °C day/14 °C night) at Bagheria, near Palermo (lat. 38°5' N, long. –13°30' E, alt. 23 m above sea level) on the coastal region of northwestern Sicily.

Mother plants of the Thai cultivars *Nguyen Muang* and *Porn Ying Yai* were grown in polypropylene benches (720 L) filled with two growing media composed of 1 coconut coir dust:1 perlite (by volume) and 2 sphagnum peat:1 perlite (by volume) in an open-loop system with no recirculating solution. The physical properties and the chemical characteristics of the media (Table 1) were analyzed according to the methods described by De Boodt et al. (1974) and Sonneveld et al. (1974), respectively.

A split-plot experimental design with two substrates as the main plot and two cultivars as subplots with three replications and 20 plants per replication was used. Mother stock plants were transplanted in early Nov. 2004 in double rows (row spacing of 0.4 m); the final density was 6.2 plants/m². Water and nutrients were supplied by a drip system controlled by a computer (MCI-Ceo; Spagnol Greenhouse Technology, Vidor, Italy). Irrigation scheduling was performed using electronic low-tension tensiometers (Tensio-technik; Floraffit, Codognè, Italy) that control irrigation on the basis of substrate matric potential. Plants were daily fertigated at 2 L/h one to five times during the growing cycle. The duration of each fertigation was adjusted when the drainage exceeded the range of 10% to 20%. The composition (mg L⁻¹) of the supplied nutrient solution was as follows: 143 N-NO₃, 47 N-NH₄, 50 phosphorus, 200 potassium, 120 calcium, 30 magnesium, 1.2 iron, 0.2 copper, 0.2 zinc, 0.3 manganese, 0.2 boron, and 0.03 molybdenum. The pH and the electrical conductivity (EC) were maintained at 5.9 and 2.1 dS m⁻¹, respectively.

Plant height increase, plant stem diameter increase, total number (basal and lateral) of shoots per plant, number of cuttings suitable for rooting (with average length of 8 to 12 cm) harvested per plant, and fresh weight of cuttings included in this range were recorded for a 12-month period (Jan. to Dec. 2005). Water absorption was calculated from the difference between the volume of nutrient solution applied and the volume of collected drainage. Nutrients content in the root zone (uptaken by roots and retained by substrate) was determined by photometric test with Spectroquant (Merck KGaA, Darmstadt, Germany) as the difference between the concentration of each element in the given solution and in the collected drainage.

Expt. 2: Rooting. This experiment was conducted in a single-span 200 m² greenhouse covered with polymethyl methacrylate maintained at 28 °C day/14 °C night. Uniform (with average length of 8 to 12 cm, weight of 20 to 25 g, and basal diameter of 1.2 to 1.5 cm), semisoft wood cuttings of ‘Nguyen Muang’ and ‘Porn Ying Yai’ from 1-year-old stock plants (Expt. 1) were harvested in early Oct. 2005 and the cut end of the cuttings were immersed in hot water (60 °C) for 30 s to prevent latex production.
and then air-dried for 8 h. Basal end of the cuttings was treated with 0 and 4000 ppm of α-naphthalene acetic acid (NAA) powder formulation (Germon; E. Gerlach Gmbh, Lubbecke, Germany). Cuttings were placed under mist into benches (6 m × 1.2 m) filled with different substrates [perlite, 1:1 coir dust (by volume), coir dust] for rooting. Benches were bottom-heated at 22 to 23 °C and covered with a PE film, which was removed from 1100 HR to 1500 HR to reduce the temperature. Mist cycles were regulated (30 s every 120 min, from 1000 hr to 1600 hr). Rooting media were drenched every 15 d with 2 mL·L⁻¹ of propamocarb hydrochloride (65% w/w) (Previcur; Bayer CropScience AG, Milano, Italy) for fungal disease control.

The experiment was set as a split-plot design with rooting substrates, auxin applications, and cultivars set as the main plot, subplots, and subsubplots, respectively. Each replication consisted of 30 cuttings. Data on the survival rate of cuttings, rooting percentage, total number of roots per cutting, and length of all roots were collected after 60 d.

**Expt. 3: Plant growth.** This trial was carried out in a greenhouse as described in Expt. 1. Rooted cuttings of ‘Nguen Muang’ and ‘Porn Ying Yai’ were transplanted into 14-cm plastic pots filled with two growing media composed of 1 sphagnum peat:1 perlite (by volume) and 2 sphagnum peat:1 perlite (by volume) in early Jan. 2006 with a final density of 10 plants/m². Pots were placed on the raised bench with 0.5% slope from the ground and the leached solution from all containers was collected.

During culture, water, macro-, and microelements were supplied through a drip fertilization system four to eight times a day as described in Expt. 1. Irrigation scheduling was based on the same criteria adopted for Expt. 1. Plants were fed with a nutrient solution with the same composition, pH, and EC of that used in Expt. 1.

A split-plot experimental design with two mixtures as the main plot and two cultivars as subplots, with three replications, was used. There were 20 plants per replication and each plant was considered as an experimental unit. Number of survived plantlets was counted 40 d after transplanting. Plant height, plant stem basal diameter, and the number of total inflorescences (flower stalks), leaves, and shoots per plant were monthly recorded for a 6-month period (Apr. to Sept. 2006).

**Statistical analysis.** Data from each experiment were subjected to two-way (Expts. 1 and 3) and three-way (Expt. 2) analysis of variance; means were compared according to Duncan’s multiple range test using Statistica software (Version 6.0 for Windows, Statsoft Inc., Tulsa, OK). Cuttings viability, rooting, and plantlet survival percentages were arcsine transformed before the analysis, but means were presented from the nontransformed data were reported.

**Results and Discussion**

**Expt. 1.** Mother plants cultivated in 2 sphagnum peat:1 perlite (by volume) showed a similar height increase than those grown in 1 coir dust:1 perlite (by volume) (25.9 and 22.1 cm, respectively) (Table 2). Hybrids cultured in peat blend evidenced a higher increment of basal stem diameter (5.2 cm) than plants on coir dust (3.6 cm). The cultivars tested differed both on plant height increase and on stem diameter increase with ‘Porn Ying Yai’ recording superior values (30.3 and 5.1 cm for height and diameter increment, respectively) than ‘Nguen Muang’ (17.7 and 3.7 cm) (Table 2). Substrate growing response supports similar outcomes previously described (Dewir et al., 2005), in which Euphorbia milii ex vitro plantlets grew better in a medium with peatmoss plus perlite and vermiculite than in a coco peat:rice hulls mix.

An average production of 18.3 shoots/plant was obtained regardless of the growing substrates (Table 2). Average amounts of 31.6 and 5.0 shoots were achieved by ‘Nguen Muang’ and ‘Porn Ying Yai’, respectively. A highly significant (P ≤ 0.001) interaction substrate × cultivar was recorded. Differences between substrates on shoot production, during a 12-month period of cultivation, varied from 1.3 to 5.3 shoots/plant only, whereas those measured between cultivars ranged from 5.9 to 23.9 shoots/plant (February) (Fig. 1).

A higher number (10.4) of cuttings suitable for rooting was produced from plants cultivated in peat-based substrate compared

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**Table 1. Physical and chemical characteristics of the growing media used in Expt. 1.**

| Media Type         | Bulk density (g·cm⁻³) | Total pore space (% vol.) | Air content (% vol.) | Waterholding capacity (mL·g⁻¹) | pH (H₂O) | Electrical conductivity (dS·m⁻¹) | Cation exchange capacity (meq/100 g) |
|--------------------|-----------------------|----------------------------|----------------------|---------------------------------|-----------|---------------------------------|-------------------------------------|
| 1 coir dust: 1 perlite | 0.11                  | 95.3                       | 48.1                 | 47.2                             | 13.4      | 0.6                             | 36.1                                |
| 2 peat: 1 perlite   | 0.12                  | 93.1                       | 34.5                 | 58.6                             | 20.1      | 0.5                             | 53.2                                |

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**Table 2. Effects of substrates and cultivars on Euphorbia ×lomi plant height and diameter increase, shoot production, cutting production, and fresh weight (Expt. 1).**

| Substrate                        | Plant ht increase (cm) | Plant stem diam increase (cm) | Shoots* (no./plant) | Cuts* (no./plant) | Cutting fresh wt (g) |
|----------------------------------|------------------------|-----------------------------|---------------------|-------------------|----------------------|
| 1 coir dust: 1 perlite           | 22.1 a                 | 3.6 b                      | 15.5 a              | 5.6 b             | 23.6 a               |
| 2 peat: 1 perlite                | 25.9 a                 | 5.2 a                      | 21.0 a              | 10.4 a            | 26.8 a               |
| Cultivar                         |                        |                             |                     |                   |                      |
| Nguen Muang                      | 17.7 b                 | 3.7 b                      | 31.6 a              | 13.1 a            | 20.5 b               |
| Porn Ying Yai                   | 30.3 a                 | 5.1 a                      | 5.0 b               | 2.9 b             | 30.0 a               |

**Significance**

| Substrate (S) | NS | NS | NS | NS |
|---------------|----|----|----|----|
| Cultivar (C)  | **| **| **| **|
| S × C         |    |    |    |    |

**NS, *, **, ***: Non-significant or significant at P ≤ 0.05, 0.01, or 0.001, respectively.**

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**Fig. 1. Monthly productive trend of the two Euphorbia ×lomi hybrids on soilless culture with two organic media (Year 2005; Expt. 1).**

- 1 coir dust: 1 perlite (by volume).
- 2 sphagnum peat:1 perlite (by volume).

Vertical bars indicate ± SE of means.
with that (5.6) from coir-grown hybrids (Table 2). Average harvest of 13.1 and 29.6 cuttings/plant were recorded for ‘Nguen Muang’ and ‘Porn Ying Yai’, respectively. A significant (P ≤ 0.05) interaction substrate × cultivar was observed. Hybrids in the two growing substrates produced cuttings with similar average fresh weight (23.6 and 26.8 g for plants in coir dust and sphagnum peat, respectively) (Table 2); ‘Porn Ying Yai’ provided heavier cuttings (30.0 g) than those supplied by ‘Nguen Muang’ (20.5 g).

A higher water absorption was recorded from plants grown in sphagnum peat mixture (324.3 and 206.2 mL/plant/d, for spring/summer and fall/winter, respectively) than those cultivated in 1 coir dust:1 perlite (by volume) (189.3 and 117.5 mL/plant/d) (Table 3). There was no statistical difference in water absorption between cultivars. Plants in 2 perlite (by volume) showed higher macro- and micronutrient content in the root zone compared with that measured in coir dust blend (Table 4). The two hybrids did not differ on this parameter.

Effects of the two organic mixes on plant growth, water and nutrient absorption are certainly related to their physical and chemical characteristics, which were formerly studied by several authors (Evans et al., 1996; Prasad and Mahler, 1993; Verdonck et al., 1983; Wever, 1995) who reported that the two growing materials, even presenting some similarities, mostly differ on important chemical and hydrofugal properties; coir dust is characterized by higher porosity and air content and lower total and easily available water capacity than peat (Abad et al., 2005). In our trial, the higher diameter increase and the superior production of cuttings by plants in 2 sphagnum peat:1 perlite (by volume) may be correlated to its higher water content that, together with a sufficient degree of aeration, not too far from that of coir dust blend, could establish better environmental conditions in the root zone, which was also characterized by superior content of macro- and micronutrients (in the roots as well as in the substrate). Nevertheless, with appropriate adjustments of water and mineral regimes, their performances may be equivalent.

<Table 3. Effects of substrates and cultivars on seasonal water absorption of Euphorbia x lomi plants (mL/plant/d; Expt. 1).>

| Substrate           | Spring–summer | Fall–winter |
|---------------------|---------------|-------------|
| 1 coir dust:1 perlite | 189.3 b     | 117.5 b     |
| 2 perlite:1 perlite  | 324.3 a      | 206.2 a     |
| Cultivar            |               |             |
| Nguen Muang         | 250.7 a      | 157.1 a     |
| Porn Ying Yai       | 262.9 a      | 166.6 a     |
| Significance         |               |             |
| Substrate (S)       | **            | **          |
| Cultivar (C)        | **            | **          |
| S × C               | NS            | NS          |

*Means within columns separated using Duncan’s multiple range test (P ≤ 0.05).

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(Meerow, 1994). Actually, no differences recorded on plant height increase, shoot total production, and quality of cuttings seem to indicate that Euphorbia x lomi hybrids can be grown in both the substrates, confirming coir dust as one of the main alternatives to peat as an organic component of mixtures for hydroponic culture (Cresswell, 1992; Evans and Stamps, 1996; Stamps and Evans, 1999). Expt. 2. An average value of 83.3% of viable cuttings was obtained irrespective of the rooting substrates (Table 5). The application of NAA did not affect the survival rates of the cuttings. ‘Nguen Muang’ showed higher cutting viability (91.0%) than ‘Porn Ying Yai’ (75.5%). Significant (P ≤ 0.05) two-way (substrate × cultivar) and three-way (substrate × hormone × cultivar) interactions were observed.

Higher rooting rates were recorded from cuttings inserted in perlite-based media [86.2% and 76.2% for perlite and 1 perlite:1 coir dust (by volume), respectively] than from coir dust alone (58.3%) (Table 5). Application of NAA resulted in a higher number of rooted cuttings than nontreated cuttings (78.8 versus 68.3). Cuttings of ‘Nguen Muang’ showed a higher rooting percentage (79.0%) compared with ‘Porn Ying Yai’ (67.0%).

Cuttings in perlite and 1 perlite:1 coir dust (by volume) produced a higher number of roots (14.5 and 13.5, respectively) than those inserted in pure coir dust (10.9) (Table 5). NAA treatment increased the number of roots (13.4) over control (10.2). ‘Nguen Muang’ produced a higher number of roots/cutting (14.6) than ‘Porn Ying Yai’ (11.1).

Cuttings in perlite presented longer roots (6.2 cm) than those in the other media [4.1 and 3.5 cm for 1 perlite:1 coir dust (by volume) and coir alone, respectively] (Table 5). Root length was unaffected by NAA treatment and by cultivars.

The highest rooting percentage and roots production recorded with perlite-based media suggested that coir dust alone is less suitable for Euphorbia x lomi propagation, probably because of the establishment of less favorable environmental conditions for the development of root organ. The best crop performances in perlite are in line with other researches according to which rooting response of Clematis microshoots and stem cuttings was higher in pure perlite than in peat/perlite (Kreen et al., 2002). Al-Salem and Karam (2001) reported that adventitious root formation of Arbutus andrachne stem cuttings was enhanced with increasing perlite percentage in the medium up to 100%. The
significant differences among the three rooting media are probably related to the physical properties of substrates, which are characterized by different levels of porosity and waterholding capacity (Hartmann et al., 1997). The improvement of the formation and growth of roots of *Euphorbia ×lomi* cuttings in pure perlite could be linked to a likely increase of air content and oxygen diffusion rate than in coir-based media as reported by Gislerød (1983) for Poinsettia’s cuttings in coir dust and rockwool.

Higher NAA effect on rooting rate and number of roots produced by each cutting than treatment without auxin agrees with previous reports describing the effectiveness of NAA in promoting adventitious root formation (Loach, 1988; Lym, 1992).

**Expt. 3.** Higher survival rate (84.5%) of potted plants was observed in 1 sphagnum peat:1 perlite mixture than 2 sphagnum peat:1 perlite (by volume) mixture than 2:1 medium (4.4) (Table 6). No differences on shoot production were observed between hybrids.

### Conclusion

The definition of a vegetative propagation protocol that allows a fast and low-cost mass production of pot plants of *Euphorbia ×lomi* cultivars could be exploited in commercial contexts aimed to propose new floricultural crops. These hybrids may be seriously considered as a valid alternative to the traditional potted plants, considering the high ornamental potentialities (great size inflorescence, long-lasting flowering, water deficit resistance) and taking into account the renewed general interest on drought-tolerant and easy-maintenance species. Additional studies should be conducted to increase plant growth performances and cutting rooting response by testing different growing media, auxin types, and formulations.

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