treatments.

While both positive (1, 11, 12) and negative (7) data exist correlating element uptake and media moisture tension, there seems to be more support for a positive correlation viz., as available media moisture increases, element uptake increases (11). Media amended with VH increases water availability to plant roots (3, 6), thus, theoretically enhancing element uptake increases (11). However, based on data obtained in the present study, foliar element levels of chrysanthemums and tomatoes were not consistently altered by the VH-amended media in which they were grown.

While no conclusive explanation of these data will be presented, the presence of VH in media may influence: 1) mobility of media solution; 2) continuity of moisture films; 3) solvent contact exchange between root and media surfaces; 5) oxygen diffusion rates; and/or 6) contact exchange between root and media surfaces. All of these conditions are known to influence element uptake as related to media moisture tension (1, 2, 11, 13, 15).

Literature Cited


Carnation Propagation from Shoot Tips Cultured in Liquid Medium1

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Abstract. Shoot tips of carnations (Dianthus caryophyllus L. cv. CSU White Pikes Peak) formed multiple shoots on agar nutrient medium containing 0.5 mg/liter kinetin and 0.1 mg/liter 2-naphthaleneacetic acid. Tissue with shoots was transferred to liquid medium on a culture wheel rotating 1 rpm. Many axillary shoots formed and eventually separated from the parent shoot. Tissue could be subcultured into fresh medium, stored at 4.5oC, or rooted, potted and grown to flowering that the chimeral arrangement of the petal tissues had not been disturbed by the culture procedure.

Production of virus-free carnations by “meristemming” is a well-established procedure (5) in which one excised shoot tip is grown into one plant. A tissue culture technique for producing many plantlets from a single shoot tip was described by Hackett and Anderson (3) but their method has not been commercially adopted, perhaps because some plantlets produced from “White Sim” cultures had red flowers. We present here a simple aseptic technique for growing one shoot tip into many plantlets which all flower true. The procedures are similar to those successfully used for propagation of Chrysanthemum in vitro (1, 2).

Shoots were broken off stock plants of ’CSU White Pikes Peak’ carnations grown in perlite, and use leaves longer than 5 mm were removed by hand. Smaller leaf primordia, axillary buds, and any remnants of larger leaves were gently scraped off with a sterile dissecting needle or scalpel. Explants for culture consisted of the apical dome plus subjacent tissue; they were ca. 0.5 mm high and 0.5-1.0 mm wide at the base. No surface sterilization was needed.

Explants were placed apex up on nutrient medium in plastic petri dishes sealed with Parafilm. In addition to Murashige-Skoog inorganic components (4), the medium contained 100 mg/liter myo-inositol, 0.4 mg/liter thiamin-HCl, 30 mg/liter sucrose, 6 g/liter Bacto-agar and varying levels of plant growth hormones. pH was adjusted to 5.7 before autoclaving. Cultures were kept at 25°C with continuous cool white fluorescent light of 1-4 klx.

Medium containing 2.0 mg/liter 2,4-dichlorophenoxyacetic acid (2,4-D) suppressed leaf and shoot development and caused formation of friable callus (Fig. 1). This callus grew rapidly and was easily subcultured. Roots formed on the subcultured callus when the auxin level was reduced, but no buds were seen. Both callus and roots grew on explants grown with 2.0 mg/liter NAA, but shoot growth was usually suppressed.

On media containing combinations of kinetin (0-2.0 mg/liter) and NAA (0-0.5 mg/liter), shoots formed on the top of the explants while callus and sometimes roots developed from the base (Fig. 2). NAA promoted rooting and callus formation; 2.0 mg/liter kinetin slowed growth and reduced shoot size. Development of vigorous multiple shoots was most consistent when the medium contained 0.5 mg/liter kinetin and 0.1 mg/liter NAA.

Fig. 1. Carnation shoot tip after 4 weeks growth on medium containing 2.0 mg/liter 2,4-D. (1X)

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Formic acid (10 mg/liter GA₃) strongly promoted shoot elongation, but because plantlets were spindly and weak, GA₃ was not routinely added to the nutrient medium.

After 3-6 weeks growth on agar medium, whole explants with multiple shoots or portions of such explants were transferred to 500 ml Erlenmeyer flasks containing 30 ml of liquid medium. The medium contained 2.0 mg/liter kinetin and 0.02 mg/liter NAA (a combination which had been effective with chrysanthemum cultures (1, 2) or in some cases 0.5 mg/liter kinetin and 0.1 mg/liter NAA. Flasks were held horizontally on a culture wheel rotating 1 rpm.

In liquid, many axillary shoots usually developed. Most of them eventually broke off and floated free in the medium. These shoots in turn formed new axillaries, so growth in liquid medium was rapid. One explant had produced 59 clumps of shoots 6 weeks after transfer from agar medium.

Some of the shoots were used to start new liquid cultures, one of which has been maintained since April, 1973. Flasks inoculated with 5 small shoots contained an average of 28 separate tissue clumps after 4 weeks and 63 after 5 weeks. The rate of increase is not a simple geometric one since to be counted, a shoot must both develop and detach from the original shoot. Thus, the total no. of shoots present in a flask exceeded the no. of clumps.

Fig. 3 shows the increase in tissue in one flask after 5 weeks. The material produced ranged from large clumps with several shoots to very small single shoots (Fig. 4). Shoots were seldom longer than 1-2 cm because medium containing kinetin encouraged production of axillaries rather than shoot elongation.

Many subcultured shoots formed roots, either spontaneously while in liquid medium or within 2 weeks after transfer to agar medium lacking hormones (Fig. 5). Large clumps and shoots with thickened leaves usually turned brown and failed to root on agar.

Small (usually 5-20 mm high) plantlets were potted in Jiffy-Mix or 1 vermiculite:1 perlite (v/v). They were grown in a 20°C growth chamber with a 16 hr day and 10,000 lux of mixed fluorescent and incandescent light. Until they were well established, plantlets were covered with a polyethylene tent to keep humidity high.

Some shoots and plantlets were rooted or potted after several weeks storage at 4.5°C. Refrigeration was a convenient way to store material that otherwise would have required transfer to fresh medium.

Approx 45% of the rooted shoots survived the transfer from culture. Survival was higher (75%) among plantlets which were 20 mm high when...
Fig. 4. Selected pieces from the plate in Fig. 3, showing small single shoots, large single shoots, and clumps with many shoots.

Fig. 5. Root formation on carnation shoots after transfer from liquid medium to agar medium containing no hormones.

Fig. 6. Plantlets in flat after transfer from agar medium.

Fig. 7. Sequence of plants from initial transplant to flowering.

grown to flowering in the growth chamber or the greenhouse (Fig. 7). Some plants showed fasciation and excessive shoot formation at the base, but one or more normal shoots grew up from each plant. Time from potting to flowering averaged 6 months. A total of 175 plants have flowered, all with normal white flowers and the occasional small red flecks typical of the cultivar ‘CSU White Pikes Peak’; 300 cuttings taken off plants derived from cultures rooted, grew and flowered normally. Plants from ‘Pink Sim’ and ‘Red Sim’ cultures produced normal flowers of the appropriate colors.

This procedure gives a high rate of multiplication in vitro. Four months of proliferation in liquid (assuming a 60 fold increase every 6 weeks) might produce 100,000 flowering plants from one shoot tip within a year. A key feature of the system is the use of culture flasks on a slowly rotating wheel. Under these conditions, new shoots spontaneously detach from the inoculum, so that little cutting is required for transfers. Since no red flowers were produced and the white petals all showed small red flecks, the chimeral arrangement of the petal tissues was apparently not disrupted in culture. This stability is probably related to the fact that the new shoots are all axillary buds and are not formed from callus tissue.

With improvements in media and transplanting procedures, this technique could be used for rapid production of many virus-free plants from a single shoot tip excised from a disinfected (possibly heat-treated) plant. Chances of reinfection during in vitro culture would be small.

Literature Cited


