

the salt content to undesirable levels, and are not likely economical amendments.

Additions of peat moss (pH 3.4) to compost in 1:1 and 3:1 ratios lowered the pH to 5.3 and 4.4. The pH of the mixture continued to increase with

time, however. Additions of soils of low pH also gave similar results. Merrimac soil used in Table 2 had a pH of 4.8. The resistance to change in pH is most likely due to buffering action of the organic acid components of the compost.

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Tissue Culture Propagation of the Double *Petunia*¹

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Abstract. Leaf pieces of pistil-sterile double petunias (*Petunia hybrida* Hort.) placed on a Linsmaier-Skoog (LS) medium containing 0.2 mg/liter 6-benzylamino purine (BA) produced shoots after 4 weeks which rooted within a week when the cut ends were dipped into an LS medium containing 1.0 mg/liter naphthalenacetic acid (NAA) and then placed in a hormone-free medium. Plantlets grew rapidly after transfer to soil and had similar growth and flower type as the source clone.

Double petunias form multi-petaled flowers which are desirable in the bedding plant industry. The flowers are pistil-sterile, therefore, double petunia seeds are produced by using the double petunia to pollinate a single-petaled petunia (3). The double petunia pollen parent can be vegetatively propagated, but it is slow to root by stem cuttings and susceptibility to tobacco mosaic virus leads to serious problems. Rao et al. (5, 6) were able to propagate single-flowered cultivars of the petunia by placing stem segments or leaf discs on a medium containing macroelements of Murashige and Skoog's (2), the microelements and vitamins of Nitsch and Nitsch (4), 2% sucrose, 0.2 mg/liter BA, and 0.8% agar. On this medium shoots formed from the cut surfaces which were later excised and rooted on the above medium but lacking BA. In this paper we describe a similar method for culture of the double petunia.

Double petunia clones (DS2, DS4, and DP1) were obtained from George J. Ball, Inc., West Chicago, Ill. Fully

expanded and young leaves were excised, surface sterilized for 10 min in a 0.5% sodium hypochlorite (10% Chlorox) solution to which a few drops of Tween 20 had been added, then rinsed for 5 min in sterile distilled water and cut into 2 to 12 pieces with a scalpel. The pieces were aseptically transferred to petri plates containing LS (1) medium to which 0.2 mg/liter BA and 0.5% agar had been added (pH 5.7–5.8). The petri plates were sealed with Parafilm and kept under continuous light (approx 1000 lux) at approx 25°C. Transfers to fresh medium were made every 2 weeks.

Shoots which formed were excised and rooted on one of the following media: 1) LS without hormones, 2) LS with 1.0 mg/liter NAA, or 3) LS with 0.1 mg/liter NAA. As an additional treatment, the cut ends of some shoots were dipped in liquid LS medium containing 1.0 mg/liter NAA and then transferred in the hormone free medium. After roots had formed, the plantlets were removed from culture, planted in Jiffy mix, and grown to flowering in the greenhouse.

Callus developed around the cut edges soon after the mature leaf pieces were placed on the medium, but turned yellow after 10 days, and all died after a few weeks. The young leaf pieces expanded in size, thickened, and turned

darker green within a week after being placed on the medium. A little callus formed on the cut edges from which shiny green nodules later appeared. After 2 weeks of culture, shoots developed from the shiny green nodules. Within 4 weeks some of the shoots were excised and rooted. Of the 49 pieces cultured, 20 became contaminated with various kinds of bacteria and of the non-contaminated, 13 formed shoots. The 3 cultivars tested performed similarly.

Some rooting took place with each of the 4 root promoting treatments. Shoots left on the LS medium without hormones developed roots slowly and sometimes, not at all. Those left on the NAA containing medium formed large white calli on the cut ends of the shoots from which a few thick roots developed. Shoots whose cut ends were only dipped in the NAA-containing medium formed almost no callus but within a week, abundant roots appeared. Rooted plantlets which were potted survived and grew rapidly into plants appearing identical in flower color and form to the parents.

This method of culturing offers a simple technique to asexually propagate the female-sterile petunia.

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