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In Vitro Shoot Formation and Elongation of Dwarf Pomegranate

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Dwarf pomegranate *(Punica granatum* L. 'Nana') is used for landscape, indoor culture, and bonsai. Adventitious shoot formation has been reported in anther and leaf callus cultures of tree pomegranate (Moriguchi et al., 1987; Omura et al., 1987). In vitro propagation has also been reported for tree pomegranate (Mascarenhas et al., 1981). However, we found no information on in vitro propagation of dwarf pomegranate. In this paper, we report in vitro shoot formation from nodal sections of dwarf pomegranate.

Terminal shoots with seven to 10 nodes were excised from actively growing plants of dwarf pomegranate maintained in a greenhouse. Shoots were surface-disinfected as described previously (Zhang et al., 1987) and then cut to provide nodal sections (≈ 5 mm long). The explants were placed vertically in 25 × 100 mm culture tubes each containing 10 ml modified MS medium (Murashige and Skoog, 1962; Zhang et al., 1987). For shoot formation and elongation, the medium was supplemented with the following combinations of plant growth regulators: 1) with the NAA (1-naphthaleneacetic acid) level fixed at 2.0 µM, BA [*N* -(phenylmethyl)-l *H* purin-6-amine] levels were 0, 0.5, 1.0, 2.0, 4.0, 8.0, or 16.0 µM; and 2) with the BA level fixed at 2 µM, NAA levels were 0, 0.5, 1.0, 2.0, 4.0, 8.0, or 16.0 µM. The experiment consisted of 14 treatments: two factors (BA and NAA) each with seven levels. A treatment had 20 explants each in a culture tube. Medium pH was adjusted to 5.8 before autoclaving at 120C for 20 min. All cultures were incubated at 25 ± 1C with a 16-h photoperiod from cool-white fluorescent lamps that provided a photosynthetic photon flux of 50 $\mu mol \cdot s^{-1} \cdot m^{-2}$ at explant level as measured with a LI-185B photometer (LI-COR, Lincoln, Neb). Shoots ≥ 5 mm long were recorded 6 weeks after the explants were placed on their respective media.

Each culture produced a mean of 5.2 shoots with 1.0 µM BA with NAA fixed at 2.0 µM (Fig. 1A). BA at other concentrations resulted in fewer shoots, and at higher levels callus was produced. Omura et al. (1987) also found BA at 1.0 µM to result in high shoot production from leaf callus of tree pomegranate. There were 6.6 shoots produced per culture with 1.0 µM NAA with BA fixed at 2.0 µM (Fig. 1B). NAA at other concentrations resulted in lower shoot production, and at higher levels callus was produced. We observed microscopically that shoot formation resulted from axillary bud proliferation.

Shoots were >35 mm long at BA levels ≤ 1.0 µM with NAA fixed at 2.0 µM (Fig. 1A). At BA levels 22.0 µM, shoot length was <20 mm. Shoots were 16 to 24 mm long at NAA levels ≤3.0 µM with BA fixed at 2.0 µM (Fig. 1B). At 8.0 µM NAA, shoot length was 8 mm. Therefore, shoot elongation decreased with BA and NAA concentrations > 1.0 and 3.0 µM, respectively.

For tree pomegranate, Mascarenhas et al. (1981) used MS medium supplemented with ($mg \cdot liter^{-1}$) 0.5 (2.3 µM) kinetin [*N* -(2-furanylmethyl)-1 *H* -purin-6-amine] plus 1.0 (4.5 µM) BA; no auxin was included in their shoot proliferation medium. Our work showed that auxin (NAA) at 1.0 µM is optimal for in vitro shoot proliferation of dwarf pomegranate and that BA levels > 2.0 µM inhibit shoot formation.

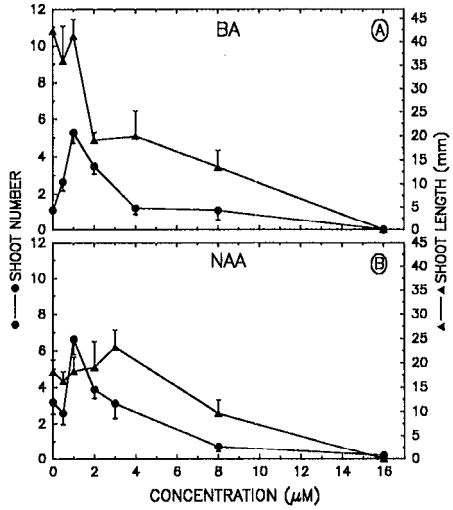

Fig. 1. Effect of BA (A) and NAA (B) concentration on in vitro shoot formation and elongation of *Punica granatum* 'Nana' on MS medium supplemented with either 2.0 µM NAA (A) or 2.0 µM BA (B). Each data point represents the mean ± 1 SE of 20 cultures.

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