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## Induction of Morphogenic Callus Cultures from Leaf Tissue of Garlic

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**Abstract.** Morphogenically regenerable callus was induced from young leaf and meristem tissues of garlic (*Allium sativum* L. cv. Howaito-Roppen). Five auxins were compared for their ability to induce morphogenic callus. In order of decreasing effectiveness, 2,4-D (0.1–3.0 mg·liter<sup>-1</sup>), 2,4,5-T (0.3–10 mg·liter<sup>-1</sup>), dicamba (10–30 mg·liter<sup>-1</sup>), and picloram (10–30 mg·liter<sup>-1</sup>) were capable of morphogenic callus induction, while NAA did not induce morphogenic callus formation over a wide range of concentrations. The morphogenic callus was nodular and gave rise to plantlets following transfer to medium containing BA. Chemical names used: (2,4-dichlorophenoxy)acetic acid (2,4-D); (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T); 3,6-dichloro-2-methoxybenzoic acid (dicamba); 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid (picloram); 1-naphthaleneacetic acid (NAA); and *N*-(phenylmethyl)-1*H*-purin-6-amine (BA).

Improvement of garlic (*Allium sativum* L.) through classical breeding techniques is not possible because cultivated garlic is sexually sterile. Standard vegetative reproduction of this crop has resulted in low propagation rates and the transmission of virus diseases. For this reason, *in vitro* techniques have been developed for garlic. Callus culture and plant regeneration (1, 5, 7), stem tip culture (9), shoot proliferation (2), and cold preservation of important germplasm (3) have been re-

ported. In spite of the numerous publications on tissue culture of garlic, basic protocols have been rather limited. Previous reports used media containing a mixture of growth regulators (auxin and cytokinin) and stem tips as explant sources. This report demonstrates that young leaf tissue can also be a source of regenerable callus. The use of leaf tissue as a explant source is desirable because many explants can be derived from a single shoot, whereas only one stem tip can be obtained from a single shoot or clove. Regenerable callus was induced by a single addition of several synthetic auxins, some of which have never been evaluated in a garlic tissue culture system.

*In vitro* plantlets regenerated from a proliferating shoot culture of a commercial Japanese garlic, 'Howaito-Roppen' (six white cloves), were used as the explant source in this study. Initially, stem tip-derived callus was initiated and maintained on a shoot proliferation medium [modified from Oosawa et al. (8)] containing Murashige and Skoog (MS) salts (6), Gamborg's B5 vitamins (4), 1 mg NAA/liter, 2 mg BA/liter, 30 g sucrose/liter

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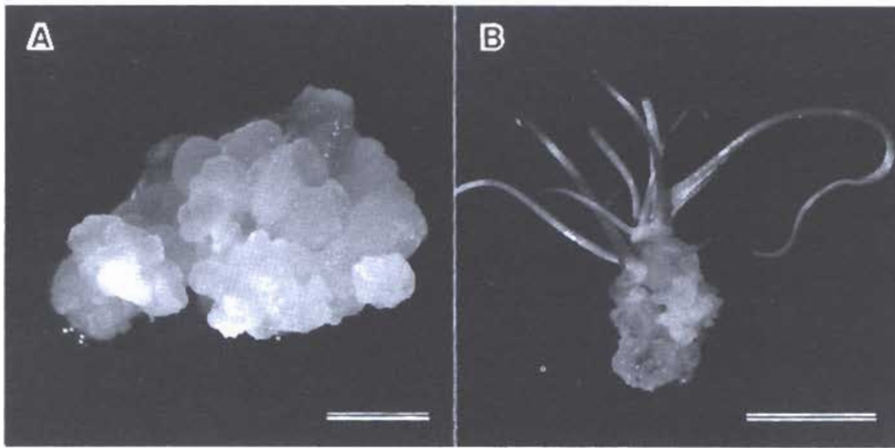


Fig. 1. **A)** Morphogenic callus induced from a leaf section of garlic cultured on medium with 10 mg dicamba/liter (Bar = 2 mm). **B)** Shoot regeneration from morphogenic callus following transfer to a hormone-free medium (Bar = 10 mm).

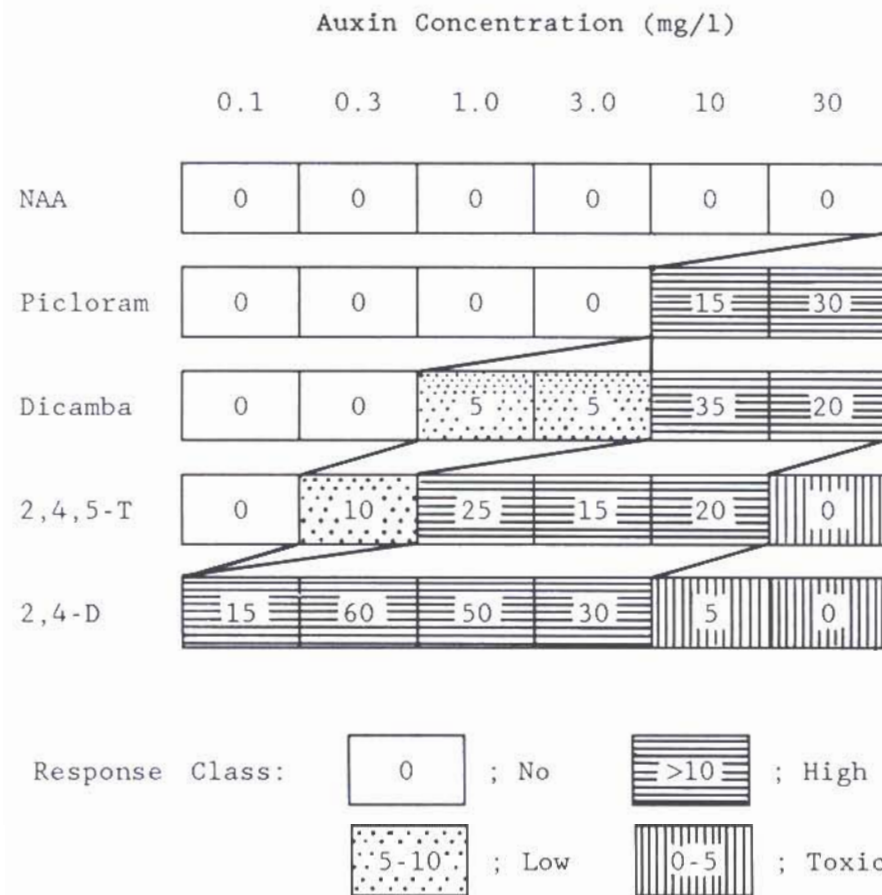


Fig. 2. Effect of different auxins and their concentrations (in mg·liter<sup>-1</sup>) on percent garlic leaf sections forming morphogenic callus after 4 months of culture. There was no callus induction on an auxin-free medium. Responses were divided into percent response classes; no response (0%), low response (5%–10%), high response (>10%), and toxic effect (0%–5%). (MW: 2,4-D, 221; 2,4,5-T, 255; dicamba, 221; picloram, 241; NAA, 186)

and solidified with 8 g agar/liter (pH 5.8). Multiple shoots and buds that developed on this medium were visually selected and subcultured every 2 to 3 months. For generation of *in vitro* plantlets, small shoot buds were excised and cultured on a hormone-free medium (same as the shoot proliferation medium except without hormones). After 2 weeks of culture on this medium, shoots that developed to 20 to 30 mm in length were

selected for callus induction.

The selected shoots were sliced into five 2-mm-long sections (Fig. 3, top) and placed on media containing five different auxins (2,4-D, 2,4,5-T, dicamba, picloram, and NAA) each at six concentrations (0.1, 0.3, 1.0, 3.0, 10, and 30 mg·liter<sup>-1</sup>). The basal section will be referred to as the meristem section as it contains the apical meristem, while the apical four sections will be referred to as leaf

sections. For each treatment, 25 sections from 25 sections from five shoots were placed on the medium in a petri dish and cultured at 28°C under 16:8 hr light:dark photoperiod with a light intensity of 30 μmol·s<sup>-1</sup>·m<sup>-2</sup>. Additionally, in order to evaluate the positional effect of shoot sections on callus induction, 150 sections from 30 shoots (six dishes for each treatment) were inoculated onto media containing the various concentrations of 2,4-D. After 2 months of culture, all tissues were transferred onto the same medium (used for initiation) and cultured for an additional 2 months. The induced callus tissues were then transferred onto either the shoot proliferation medium (with NAA at 1 mg·liter<sup>-1</sup> and BA at 2 mg·liter<sup>-1</sup>) or the hormone-free medium for plant regeneration.

Within a week after inoculation, shoot elongation followed by rooting was observed in some of the cultures from the meristem section of the shoot. This meristem-originated response was common on media containing either no growth regulators or (all in mg·liter<sup>-1</sup>) less than 3.0, NAA; 1.0, picloram; 1.0, dicamba; 0.3, 2,4,5-T; or 0.3, 2,4-D. These shoots were apparently formed from either the preexisting shoot apex or the axillary buds and did not represent *de novo* shoot regeneration as no shoot formation was observed from cultures of leaf sections.

Callus formation from the meristem section was observed after 2 weeks of culture. The response was slower from the leaf sections, where callus was initiated only after a minimum of 3 to 4 weeks of culture. However, at the end of the second transfer period on the induction media, there seemed to be no noticeable size difference in callus tissues originating from either meristem or leaf sections. All of these morphogenic callus tissues maintained a high level of organization; the tissue did not completely dedifferentiate to an unorganized 'callus'. The morphogenic callus proliferated as a yellow, smooth, compact, nodular tissue on the induction medium (Fig. 1A).

The percentages of leaf sections that formed morphogenic callus in response to the various types and concentrations of auxins are displayed in Fig. 2. For ease of comparison of the effectiveness of morphogenic callus induction among different auxins, the responses have been divided into percent response classes; no response (0%), low response (5%–10%), high response (>10%), and toxic effect (<5%), where high auxin levels reduced morphogenic callus induction. Although 2,4-D was the most effective auxin for the induction of morphogenic callus, 2,4,5-T, dicamba, and picloram could also induce the formation of morphogenic callus but at higher concentrations. Morphogenic callus was not induced from the leaf or meristem sections of the shoot if NAA was used as the auxin, even at the highest concentration tested. No morphogenic callus was formed if auxin was not included in the induction medium.

The effect of various concentrations of 2,4-D on induction of morphogenic callus from

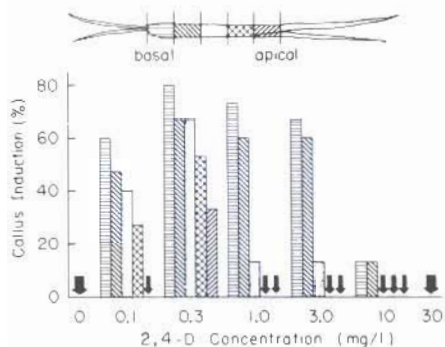


Fig. 3. Effect of 2,4-D concentration and position of shoot sections on morphogenic callus induction from garlic shoots after 4 months of culture. Arrows indicate no callus induction.

ordered shoot sections is shown in Fig. 3. This experiment gave rise to a typical dose-dependent response curve, in which 2,4-D at 0.3 mg·liter<sup>-1</sup> was chosen as optimal for all sections. The meristem section of the shoot gave the highest callus induction but the leaf sections, especially those excised from lower position of shoot, also formed morphogenic callus at a fairly high frequency by the end of induction period.

The morphogenic callus, which proliferated as organized tissue, did not give rise to shoots on the induction medium (Fig. 1A). Four weeks following transfer to either the hormone-free or shoot proliferation medium (containing NAA and BA), the nodular tissue turned green and formed numerous meristematic structures, which in turn grew to form shoots (Fig. 1B). Shoot morphogenesis has been named as the route of regeneration in all reports on garlic regeneration except one (1). Abo El-Nil (1) describes both shoot morphogenesis and somatic embryogenesis in garlic. Although garlic is a monocot, an illustration of an excised torpedo-shaped structure was presented as evidence of somatic embryogenesis in that report. Although in the present report, the numerous meristematic structures at first resembled somatic embryos, further growth of these structures indicated shoot organogenesis as the route of regeneration. Bipolar structures were never formed and shoots had to be broken from the callus for subsequent rooting.

In contrast to other reports on garlic callus culture where mixtures of growth regulators were used (1, 5, 7), a single high-activity auxin was sufficient for the induction of morphogenic callus in this study. Although each of the auxins tested had different activity optima, the morphogenic calli produced in all the cases were regenerable and similar in morphology. The morphogenic calli, which have been maintained for 1 year on the shoot proliferation medium, are still capable of regenerating shoot buds that can give rise to plants. The use of both leaf and meristem tissues cultured on media containing a single auxin greatly simplifies callus culture and micropropagation in garlic.

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