Induction of Morphogenic Callus Cultures from Leaf Tissue of Garlic

Akitsu Nagasawa and John J. Finer
Department of Agronomy, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691

Abstract. Morphogenically regenerable callus was induced from young leaf and meristem tissues of garlic (Allium porrum L. cv. Howaito-Roppenn). 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⁻¹), 2,4,5-T (0.5–10 mg liter⁻¹), dicamba (10–30 mg liter⁻¹), and picloram (10–30 mg liter⁻¹) 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-dichlorophenoxyacetic acid (2,4-D); 2,4,5-trichlorophenoxyacetic acid (2,4,5-T); 3:6-dichloro-2-methoxybenzoic acid (dicamba); 4-amino-3,5,6-trichloro-2-pyridinacetic acid (picloram); 1-naphthaleneacetic acid (NAA); and 3-N-(phenoxymethyl)-4-methyl-3-carboxybenzoic acid (BA).

Improvement of garlic (Allium porrum L.) through classical breeding techniques is not possible because cultivated garlic is sexually sterile. Standard vegetative reproduction of this crop has resulted in low propagating rates and the transmission of viral diseases. For this reason, in vitro techniques have been developed for garlic. Callus cultures and plant regeneration (1, 5, 7), stem tip culture (8), shoot proliferation (9), and cold preservation of important germplasm (3) have been reported. In spite of the numerous publications on garlic culture of garlic, basic protocols have been rather limited. Previous reports have mentioned 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 an 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 clone. 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 proliferating shoot culture of a commercial Japanese garlic, 'Himawairo-Roppenn' (white clove), 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 Going et al. (7)) containing Murashige and Skoog (MS) salts (6), Gamborg's BS vitamins (4), 1 mg NAA/liter, 2 mg BA/liter, 30 g sucrose/liter.

Received for publication 25 Jan, 1988. Salaries and research support were provided by Swinerton Chemical Co., Ltd. and by NSF and Federal funds appropriated to OSU-AGEC, OARC Journal Article by 19-8. We wish to thank Barbara Nelles and Martha Tinkler for their technical assistance. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, these pages therefore must be hereby marked advertisements solely to indicate this fact.

*Visiting Scientist, present address: Saitama Chemical Co., Ltd., Biotechnology Laboratory, Takasaki Research Center, 4-3-7 Takasakagemachi, Takasaki-shi, Hyogo 665, Japan.
Auxin Concentration (mg/l)

0.1 0.3 1.0 3.0 10 30

NAA 0 0 0 0 0 0

Picoluram 0 0 0 0 0 0

Dicamba 0 0 0 0 0 0

2,4,5-T 0 20 40 15 20 0

2,4-D 15 60 50 30 0 0

Response Class: High; Low; Toxic

Fig. 2. Effect of different auxins and their concentrations (mg l-1) on percent garlic leaf sections forming morphogenic cultures after 4 months of culture. There was no callus induction on an auxin-free medium. All responses were divided into four treatment classes: no response (0%), low response (0-10%), high response (>10-50%), and toxic effect (>50%). (MW: 2,4-D, 2,4-D, 2,4,5-T, dichlorophenoxyacetic acid (DPA); NAA: 0.1, 0.3).

and solidified with 8% agarose (pH 5.8). Multiple shoots and buds that developed on this medium were visually selected and sub-cultured every 2 to 3 months. For generation of in vitro plants, small shoot buds were excised and cultured on a hormone-free medium (same as the shoot proliferation medium). After 2 weeks of culture on this medium, shoots that developed up 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, dichlorophenoxyacetic acid (DPA), and NAA) each at six concentrations (0.1, 0.3, 1.0, 3.0, 10, and 30 mg l-1). 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 five shoots were placed on one medium in a petri dish and cultured at 28°C under 16 hr light/8 hr dark photoperiod with a light intensity of 30 µmol m-2 s-1. 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 on 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 l-1) and BA at 2 mg l-1 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 l-1: less than 3.0, NAA: 1.0, picloram: 2.0, dichlorophenoxyacetic acid (DPA), 0.3, 2,4,5-T; or 0.3, 2,4-D. These shoots were separately 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 meristems or leaf sections. All of these morphogenic callus tissues maintained a high level of organization; the tissue did not completely degenerate to a state resembling "callus." The morphogenic callus proliferated as a yellow, smooth, compact, nodular tissue on the induction medium (Fig. 1A).

The percentage of leaf sections that formed morphogenic callus is in response to the various types and concentrations of auxins are displayed in Fig. 2. For ease of comparison, the effectiveness of morphogenic callus induction among different auxins, the responses have been divided into percent response classes: no response (0%), low response (0-10%), high response (>10-50%), and toxic effect (>50%), 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, dichlorophenoxyacetic acid (DPA), and picloram could also induce the formation of morphogenic callus at higher concentrations. Morphogenic callus was not induced from the leaf or meristem sections of the shoot if NAA was present 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.

In contrast to other reports on garlic callus culture where mixtures of growth regulators were used (1, 5, 7), a single high hormone amount was sufficient for the induction of morphogenic callus in this study. Although each of the media tested had different activity optimum, 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.

Literature Cited