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Short communication

Development of morphogenic suspension cultures of garlic (*Allium sativum* L.)

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Abstract. Rapidly growing, regenerable suspension cultures were obtained from meristem-derived callus cultures of garlic (*Allium sativum* L.). The liquid culture medium consisted of MS salts, B5 vitamins, 3% sucrose, 1 mg l^{-1} naphthalene-acetic acid (NAA) and 2 mg l^{-1} 6-benzyladenine (BA). The tissue in the suspension culture was yellow, smooth, organized, and proliferated as nodular clumps. Histological examination revealed that these morphogenic clumps had a well-defined epidermis. Following transfer of the morphogenic clumps to an agar-solidified medium, numerous meristems with green leaf primordia were produced.

Introduction

Cultivated garlic (*Allium sativum* L.) is sexually sterile and is therefore vegetatively propagated for commercial production. Numerous tissue culture techniques have been successfully applied to garlic. There are reports of callus culture and plant regeneration [1, 5, 7], stem tip culture [11], shoot proliferation [2], and cold preservation of important germplasm [3]. Development of suspension cultures of garlic has never been described. Although there has been one reported attempt to obtain a suspension culture of garlic [5], this attempt was not successful.

Liquid suspension cultures are generally more desirable than solid support systems due to the higher growth rate resulting from a high medium-to-tissue contact. In addition to more rapid growth, the response of suspension culture tissues to media manipulations and selection pressure is also more rapid. The establishment of a morphogenic suspension culture of garlic consisting of early-staged, organized tissue may be valuable for micropropagation and mutant selection, or as a source of regenerable protoplasts. The present report demonstrates for the first time the establishment of a regenerable suspension culture of garlic.

Materials and methods

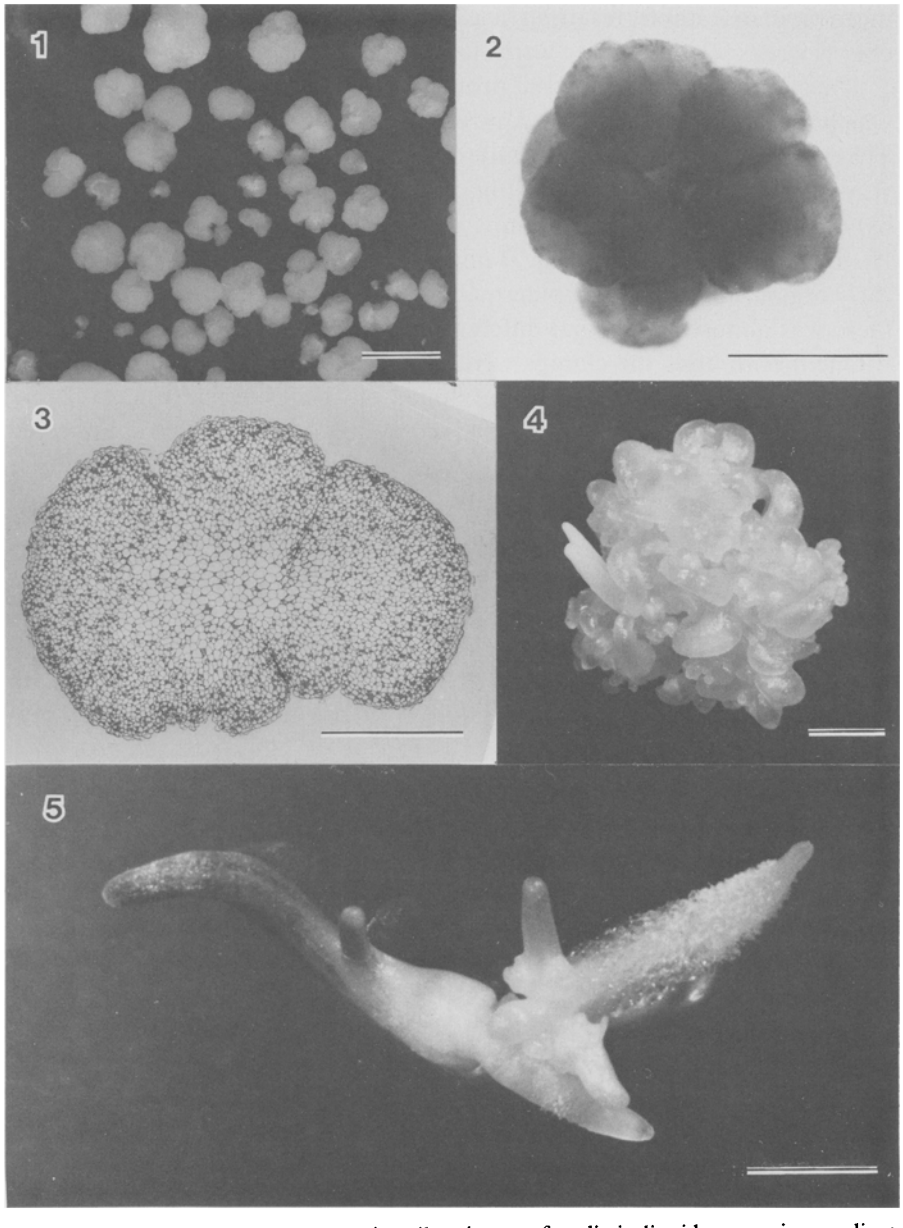
A Japanese garlic cultivar *Allium sativum* L. cv. Howaito-Roppen (six white cloves) was used in this study. Initially, stem-tip-derived callus was initiated and maintained on a shoot proliferation medium [8] containing Murashige & Skoog (MS) salts [6], Gamborg's B5 vitamins [4], $1\,mg\,l^{-1}$ NAA, $2\,mg\,l^{-1}$ BA, 3% sucrose, and solidified with 0.8% agar (pH 5.8). Multiple shoots and buds, which developed on this medium, were visually selected and subcultured every 2 to 3 months.

For induction of suspension cultures, 2 or 3 shoot buds, each 1 to 2 mm in diameter, were excised and placed in 30 ml of liquid medium. The liquid medium contained the same constituents as the shoot proliferation medium except agar was not included. All liquid cultures were agitated at 150 rpm at 28 °C with a 16/8 h light/dark photoperiod and a light intensity of $30\,\mu E\,m^{-2}\,s^{-1}$. Two months after the initiation of the suspension cultures, 40 to 60 mg of proliferating tissue were subcultured into 30 ml of fresh medium. During each subsequent 2 month subculture period, the same inoculum density was used; however, the liquid medium was removed and replaced every 2 weeks. For plant regeneration, tissues in the suspension were transferred to shoot proliferation medium with agar. Shoot buds, which developed on this medium after 2 months, were excised and transferred to a hormone-free medium (same as the shoot proliferation medium except without hormones) for further shoot and root development.

For histological studies, morphogenic tissues in suspension were fixed overnight at room temperature in a 0.2 M phosphate buffer solution (pH 7.4) containing 3% glutaraldehyde, 2% paraformaldehyde, and 1.5% acrolein. The tissues were subsequently rinsed 3 times in 0.1 M phosphate buffer (pH 7.4) and post-fixed with 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4). The tissues were then rinsed with cold water, stained overnight in 1% uranyl acetate, and dehydrated in an ethanol series. The tissues were embedded in Spurr's resin [10] and cut to 0.75 μm on a JB-4 microtome. Sections were stained with toluidine blue for light microscopy.

Results and discussion

Proliferating shoot buds of garlic, which were yellow to green in color and 1 to 2 mm in diameter, consisted of meristematic regions with organized leaf primordia. Following transfer to liquid medium, the shoot buds enlarged and turned completely yellow. Proliferation was not apparent until one month after initiation. Following this one-month lag period, growth became



Figs. 1–5 (*1*) Proliferating morphogenic callus tissues of garlic in liquid suspension medium containing 1 mg l^{-1} NAA and 2 mg l^{-1} BA (bar = 5 mm). (*2*) A single clump of proliferating tissue of garlic in suspension culture (bar = 1 mm). (*3*) A thin section of a morphogenic callus of garlic from suspension culture (bar = 0.5 mm). (*4*) Development of shoot buds from the morphogenic callus of garlic from suspension, after 1 month of culture on agar medium (bar = 2 mm). (*5*) Shoot and root elongation from excised shoot bud of garlic cultured on the hormone-free medium for 2 weeks (bar = 2 mm).

more rapid and stable, resulting in a 21.5 ± 2.6 fold increase in fresh weight over 8 weeks when cultures were started from 40 to 60 mg inocula.

The suspension culture tissue proliferated as 1 to 5 mm clumps (Fig. 1), which were nodular and less organized than the starting shoot bud material. These clumps did not contain any noticeable leaf primordia and showed very little surface organization (Fig. 2). The clumps appeared to proliferate by breakage of the larger clumps due to the agitation of the medium. Histological examination (Fig. 3) revealed that these clumps were at a very early organizational state. Epidermal cells or cell layers were developing but there was almost no internal differentiation.

One month after the clumps were placed onto solid shoot proliferation medium, numerous shoot buds developed on their surface (Fig. 4). These shoot buds possessed leaf primordia and young green leaves and appeared similar to tissue used for induction of the suspension culture. After transfer of these shoot buds to the hormone-free medium, shoots developed followed by the formation of roots (Fig. 5).

Embryogenesis in garlic has been reported in only one study [1]. Histology was not performed in that study and an illustration of an excised, torpedo-shaped structure (garlic is a monocot) was the only evidence of embryogenesis. That report also cited shoot morphogenesis as a route of regeneration. No structures resembling embryos were seen in the present report.

This report demonstrates that it is possible to obtain a liquid suspension culture of garlic comprised of partially organized, proliferating tissue, which undergoes shoot morphogenesis upon transfer to an agar-solidified medium. Due to the high medium-to-tissue contact in suspension, growth is more rapid. Response to media manipulations and selection pressures may be faster and more uniform in liquid medium than on agar-solidified medium. This morphogenic suspension culture may be a desirable system for micropropagation or mutant selection in garlic. Although embryogenic suspension cultures have been shown to be the best source for protoplast culture in monocot systems [9, 12], this early-staged morphogenic suspension culture may also be a good source of regenerable protoplasts. A protoplast system may permit, for the first time, somatic hybridization and gene transfer in garlic.

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