REGENERATION OF GARLIC PLANTS (*ALLIUM SATIVUM* L., CV. "CHONAN")

VIA CELL CULTURE IN LIQUID MEDIUM

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Summary

Aiming at the genetic improvement of garlic cultivars, a cell suspension protocol was established which includes the induction of friable callus, establishment of cells in liquid medium, plating, regeneration, and bulb formation. Calluses of various textures from compact to friable and from green to yellowish were obtained by culturing explants excised from inner leaves of garlic bulbs on Marashig-Shoog (MS) medium with 2,4 dichlorophenoxy acetic acid (2,4-D), 1.1 mg/liter [5.0 μM]), picloram (1.2 mg/liter [5.0 μM]), and kinetin (2.1 mg/liter [10 μM]). Friable callus occurred on MS-A medium containing 2,4-D alone (1.0 mg/liter [4.52 μM]) and this callus was used to develop cell suspension cultures, which were maintained in liquid MS-B medium with a 2,4-D/benzyl adenine (BA) (0.5 mg/liter [2.25 μM]: 0.5 mg/liter [2.22 μM]) ratio. High plating efficiency was obtained on MS-C medium with different naphthalene acetic acid/BA combinations. Regeneration occurred after transfer of the caulogenic mass to MS-C medium containing 10 mg/liter (74.02 μM) and 20 mg/liter (148.04 μM) adenine for 60 days, followed by transfer to adenine-free medium. Plantlets transplanted to soil showed normal phenology. Shoots grown on modified MS medium supplemented with indolylbutyric acid (3.0 mg/liter [14.7 μM]) stimulated bulb formation by 30 days in culture.

Key words: *Allium sativum* L.; cell suspension; regeneration; bulb formation.

Introduction

Traditional breeding methods are not applicable to garlic because the species produces sterile flowers and can only be propagated vegetatively (Novák and Havránek, 1975). Therefore the development of in vitro alternatives for genetic improvement of garlic cultivars would be helpful and desirable. One possibility is the use of cell suspension cultures to screen cells for genetic and physiologic characteristics, selection of desirable somaclonal variants, and introduction of foreign genes via transformation procedures. The goal of this paper was to establish a practical regeneration protocol from garlic cell suspension cultures that could be used to select new genotypes with improved, agronomically important traits.

Usually, solid medium has been used for in vitro plant regeneration of garlic (Havránek and Novák, 1976; Novák, 1983). Reports of suspension cultures, on the other hand, are uncommon in this species and even the paper by Nagasawa and Finer (1988), one of the first reports on this subject, did not report in vitro regeneration of plantlets.

Material and Methods

Stock cultures of garlic callus was used for this study. Five steps for the regeneration of plants via cell suspension were established: induction of friable callus, establishment of a liquid cell suspension, plating, regeneration, and bulb formation.

Establishment and maintenance of stock cultures. The initial explants of *Allium sativum* L. "Chonan", were obtained from proximal portions of leaves still enclosed in small bulbs and cultured on callus initiation medium containing MS salts (Murashige and Skoog, 1962), supplemented with 3.0% (wt/vol) sucrose, 0.75% (v/vol) agar, and (mg/liter) 1.1 2,4-dichlorophenoxyacetic acid (2,4-D), 1.2 4-amino-3,5,6-trichloropicolinic acid (picloram), and 2.1 kinetin (kin), adjusted to pH 5.8, and sterilized for 20 min at 1.0 kg/cm² and 120 °C. Growing callus was maintained on MS medium with 5.6 mg/liter N5-bentayladenine (BA). The cultures were illuminated with cool white fluorescent light for 16 h/day and 50 μE·m⁻²·s⁻¹ at a constant temperature of 26±2 °C. Callus cultures were subcultured at 4 wk intervals.

Friable callus induction. Compact and vigorous green stock callus was transferred to MS-A medium containing MS salts and (in mg/liter) calcium pantothenate (1.0), pyridoxine·HCl (1.0), thiamine·HCl (1.0), nicotinic acid (1.0), inositol (100), sucrose (30 000), casein hydrolysate (250), agar (7500), and 2,4-D (1.0). The callus cultures were subcultured every 4 wk.

Establishment of the suspension culture

Preparation of the inoculum. Quantities of 5 to 7 g of friable callus were transferred into 250-ml Erlenmeyer flasks containing 50 ml of MS-B medium containing MS salt with 1/2 NH₄NO₃ and 1/2 Fe-EDTA plus (in mg/liter) calcium pantothenate (3.0), pyridoxine·HCl (3.0), thiamine·HCl (3.0), nicotinic acid (3.0), inositol (100), sucrose (20 000), L-glutamine (146), 2,4-D (0.5), and BA (0.5). The growth regulators, vitamins, and L-glutamine were sterilized and added to the already cooled, autoclaved medium.

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Calluses in liquid suspension culture (MS-B) medium were incubated at 26° ± 2° C in the dark with continuous agitation at 120 rpm. A week later, the cultures were filtered through a 800-μm pore screen. The filtrates were placed in beakers and transferred to a 250-ml Erlenmeyer flask. After sedimentation of the cell, 80% of the medium was discarded and replaced by fresh MS-B, maintaining the initial volume of 50 ml. The flasks were submitted to agitation at 120 rpm during 15 days; 40% of the medium was replaced twice a week.

**Growth curves: 2,4-D/BA effect.** For the establishment of growth curves, 10 ml of cell suspension (approximately 35 mg dry weight) from stock flasks were added to 40 ml of MS-B medium in a 250-ml Erlenmeyer flask. Six combinations of 2,4-D/BA were added to the MS-B medium (in mg/liter) 0.0/0.0, 0.5/0.0, 0.0/0.5, 0.5/0.5, 1.0/0.5, and 2.0/0.5, with four replications per treatment; 40% of the medium was replaced twice a week. The pH and cell growth were monitored once a week during 5 wk. During this period the flasks were incubated as described above.

**Follow-up study of cell growth in the suspension.** The effect of different 2,4-D/BA treatments on cell growth population was assessed according to Barrueto Cid and Illg (1990), as follows: once a week, the flasks were shaken manually for 5 s approximately and placed over a ruler. Because of the agitation, the cellular sediment acquired a circular form at the bottom of the Erlenmeyer flask, and the size of the diameter was quickly measured. This procedure was done 3 times per flask, for each of the four repetitions of the treatments. Once the readings were concluded, the flasks were returned to the shaker. The statistical evaluation of the data was obtained by analysis of variance and Tukey’s test at 5% level.

**Cellular plating**

For this study the medium composition MS-C was MS salts with \( \frac{1}{2} \times \text{Fe-EDTA} \), vitamins according to MS-B, but autoclaved, and (in mg/liter) inositol (100), sucrose (20 000), casein hydrolysate (500), Difco-agar (5600), and naphthaleneacetic acid (NAA), and BA in the following combinations 0.0/0.0, 0.0/0.5, 1.0/0.0, 1.0/0.5, 1.0/1.0, and 1.0/2.0.

Five petri dishes (10 × 100 mm) per NAA/BA treatment containing 30 ml of the semisolid medium were used. Thereafter, 2 ml of cell suspension in the exponential phase of growth, 15 days old,