Water Status of Garlic Callus under Various Salt and Osmotic Stress Conditions

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Abstract. Garlic (*Allium sativum* L.) calli in vitro were evaluated over a range of salt concentrations and by adding mannitol to culture medium with reduced salt to provide equivalent osmoticum. The water potential of the medium ranged from -0.27 to -0.73 MPa under the various salt and osmotic stress conditions. The percent increase in calli was highest in standard Murashige & Skoog (MS) medium and was reduced when MS salts were reduced but the water potential of medium was adjusted to that of standard MS medium by addition of mannitol. The water potential of callus tissue was similar to that of tissue culture media over a 20-fold range (10% to 200%) of MS concentrations. Turgor of callus tissue was not influenced by any stress conditions. These results indicate that the optimum concentration of salt and water status of medium for formation of garlic calli was provided by standard MS medium.

The propagation of garlic can result in a low rate of reproduction and high rate of viral infection (Brewster, 1994). To solve these problems, many researchers have studied micropropagation of garlic plants in vitro involving a callus stage (Abo El-Nil, 1977; Fujime et al., 1993; Koch et al., 1995; Kudou et al., 1995; Nagasawa and Finer, 1988). Combinations and concentrations of plant growth regulators have been tested for the multiplication of garlic calli in vitro (Barandiaran et al., 1999; Fujime et al., 1993; Kehr and Schaeffer, 1976; Koch et al., 1995; Kudou et al., 1995; Nagasawa and Finer, 1988; Shuto et al., 1993). To our knowledge, however, the relationship between growth of garlic callus and the water status of the culture medium has not been investigated. We report how the water status of medium influences callus growth at various concentrations of salt.

Materials and Methods

Plant materials. Garlic (*Allium sativum* L. cv. Taisho) shoot tips ($3 \times 3 \times 3$ mm) were taken from cloves that had been disinfected in 1% (v/v) NaOCl solution for 10 min and rinsed twice in sterilized distilled water, and were placed on MS (Murashige and Skoog, 1962) medium in test tubes (22×100 mm, Iwaki Glass Co. Ltd., Chiba, Japan). The MS medium contained $8 \text{ g} \cdot \text{L}^{-1}$ agar (Wako Pure Chemical Ind. Ltd., Tokyo), $30 \text{ g} \cdot \text{L}^{-1}$ sucrose, $2 \text{ mg} \cdot \text{L}^{-1}$ indole-3-acetic acid and $2 \text{ mg} \cdot \text{L}^{-1}$ benzyladenine (BA). Calli formed within 2 months.

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Using the standard ratio of the chemical components for MS, we prepared media ranging from 10% to 200% (10%, 50%, 100%, 150%, and 200%) MS salts, resulting in water potentials of -0.27 to -0.73 MPa. Agar, sucrose, naphthalene acetic acid (NAA) and BA were kept at $8 \text{ g} \cdot \text{L}^{-1}$, $30 \text{ g} \cdot \text{L}^{-1}$, $2 \text{ mg} \cdot \text{L}^{-1}$, and $2 \text{ mg} \cdot \text{L}^{-1}$, respectively, for all concentrations of MS salt.

Experiments with mannitol were commenced after establishing the optimum MS concentration (100%) for the calli. Garlic calli were subcultured to a medium containing 10% MS salt + 95 mM D-mannitol (mannitol) or 50% MS salt + 44 mM mannitol. The water potential of both media was -0.48 MPa, almost the same as that for 100% MS salt + 0 mM mannitol. Additionally, a medium containing 100% MS + 53 mM mannitol, a comparable water potential to 150% MS salt without mannitol, was used. Agar, sucrose, NAA, and BA were kept at $8 \text{ g} \cdot \text{L}^{-1}$, $30 \text{ g} \cdot \text{L}^{-1}$, $2 \text{ mg} \cdot \text{L}^{-1}$, and $2 \text{ mg} \cdot \text{L}^{-1}$, respectively.

The pH of all the media was adjusted to 5.8 using 1 N NaOH. An aliquot (15 mL) of the medium was dispensed into each test tube, and then autoclaved for 15 min at 103 kPa and 121°C .

Garlic calli ($6 \times 6 \times 3$ mm) were cultured at $23 \pm 1^\circ\text{C}$ with $90 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ of photosynthetically active photon flux density and a 16-h photoperiod. About 25 calli were cultured for each treatment. Callus volume was estimated as a cylinder after measuring the height and width of the callus with a ruler, and then the percent increase was calculated by dividing the value at 0 d by that 40 d later. The increase had a linear relationship with fresh weight at 40 d after culture initiation (data not shown).

Water status as measured by isopiestic psychrometry. The water status of the callus tissues and culture media was measured using isopiestic psychrometers (model-3; Isopiestic

Psychrometry Ltd., Del.). Samples of tissues for the measurement of water status were taken from 40-d-old calli. Prior to the sampling, thermocouple chambers were coated with melted and resolidified petrolatum (Boyer, 1995). The chambers were loaded with callus tissues immediately after sampling. After the water potential measurements, the osmotic potential of the callus tissue was measured by freezing at -30°C and then by thawing (Ehlig, 1962). Turgor was calculated by subtracting the osmotic potential from the water potential. All callus tissue in an individual vessel was loaded in a thermocouple chamber for each measurement of water status. Measurements were duplicated 5–10 times for each treatment. Statistical deviations in the water status measurement with the psychrometer were evaluated by calculating the standard deviation.

Results and Discussion

When garlic calli were cultured on the media having different water potential, water potential of callus tissue had similar water potential to the culture media (Fig. 1A). The

Fig. 1. (A) Water (○) and osmotic (●) potential of callus tissue, and water potential of tissue culture medium (dashed line); (B) turgor of callus; and (C) callus increase for garlic callus grown for 40 d in tissue culture media differing by concentration of MS salt. Vertical bars indicate the standard deviation.

maximum increase of calli occurred at -0.48 MPa (100% MS salt) (Fig. 1C). Turgor of callus did not differ significantly among medium treatments (Fig. 1B).

When mannitol was used to adjust the water potential of media with reduced MS salts to that of the mannitol-free medium with 100% or 150% MS salts, the water potential of callus tissue was similar to that of callus grown in mannitol-free medium at -0.49 MPa (Fig. 2A) and at -0.60 MPa (Fig. 2D). The increase in calli was highest in the mannitol-free medium for both conditions (Fig. 2C and F). Turgor did not differ significantly among treatments (Fig. 2B and E).

Garlic callus can be induced to grow with various salt compositions of culture medium that should have different water potentials [e.g., AZ medium (Abo El-Nil, 1977); MS salts and B5 vitamins (Nagasawa and Finer, 1988); BDS medium (Koch et al., 1995); B5 medium (Barandiaran et al., 1999); MS medium (Fujime et al., 1993; Kudou et al., 1995)]. Also, different concentrations of sucrose in culture medium have been used [$20\text{ g}\cdot\text{L}^{-1}$ (Shuto et al., 1993); $30\text{ g}\cdot\text{L}^{-1}$ (Fujime et al., 1993; Koch et al., 1995; Kudou et al., 1995; Nagasawa and Finer, 1988)]. In this study, the salt content of the culture medium significantly affected the water potential and growth of tissue-cultured garlic calli (Fig. 1A and C). It might be possible that the difference of salt composition affected the growth of cultures. Thus, measurement of water status of culture media could be used to optimize conditions for tissue-cultured garlic plants.

Because mannitol is not a nutrient for plant growth, it served only to reduce the water potential of the medium with lower salt concentrations. The garlic calli did not develop well when mannitol replaced salts in the medium (Fig. 2C and F). Even when the water potential of the medium was optimum for growth but salts reduced, callus tissues were not able to develop as much as in mannitol-free medium with higher salt concentrations, suggesting that the calli were subjected to nutrient deficiency (Fig. 2C). The calli grown on 100% MS with mannitol did not develop as well as on 150% MS medium with no mannitol (Fig. 2F). This result indicates that the water status which is similar to 150% MS salt was not suitable for growth even when optimum MS concentration for growth (i.e., 100% MS) was available. Thus, MS concentration and water status of culture media could be controlling factors for growth of garlic callus.

Ikeda et al. (1999) observed that the water potential of soybean calli adapted to that of the culture medium, indicating that osmoregulation occurred and the water potential gradient between the water source (culture medium) and plant tissues (growth-induced water potential) was almost zero. In the present study, the water potential of garlic calli was similar to that of the medium when the water potential of medium was modified by salt concentration and mannitol. (Figs. 1A, and 2A and D). This suggested osmoregulation occurs in garlic callus to maintain turgor under conditions of salt stress and nutrient deficiency, and to with-

Fig. 2. (A, D) Water (○) and osmotic (●) potential of callus tissue, and water potential of tissue culture medium (dashed line); (B, E) turgor of callus; and (C, F) callus increase for garlic callus grown for 40 d in tissue culture media having (A–C) -0.48 MPa (100% MS salt + 0 mM mannitol, 50% MS salt + 44 mM mannitol and 10% MS salt + 95 mM mannitol); and (D–F) -0.60 MPa (150% MS salt + 0 mM mannitol, 100% MS + 53 mM mannitol). Vertical bars indicate the standard deviation.

stand osmotic stress induced by mannitol.

When plants are exhibiting extremely slow growth, it is possible that the medium induces a significant water stress on plants. Garlic grown in vitro takes several weeks or months before subculture (e.g., 40 d in this study; 45 d in Kehr and Schaeffer, 1976; 2 months in Abo El-Nil, 1977; 12 weeks in Koch et al., 1995; 100 d in Fujime et al., 1993; Kudou et al., 1995). Under such conditions, differences in water potential of the medium may affect growth, because cumulative effects of water stress on callus become more extreme in long-term cultures. Thus, the importance of water status in tissue culture medium should be considered when optimizing garlic in vitro propagation.

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