Stimulation of Shoot Regeneration in Spinach Callus by Gibberellic Acid

J.M. Al-Khayri, F.H. Huang, T.E. Morelock, and T.A. Busharar

Department of Horticulture and Forestry, University of Arkansas,
Fayetteville, AR 72701

Additional index words. gibberellin, leaf explant, *Spinacia oleracea*, tissue culture

In most cases, gibberellins are not essential for in vitro plant cultures and are generally not incorporated into the medium (Pierik, 1987). These plant hormones usually inhibit root (Pierik and Steegmans, 1975) and shoot formation (Appelgren and Heide, 1972; Murashige, 1964; Schraudolf and Reinert, 1959). Al-Khayri et al. (1991a, 1991b) reported on the lack of shoot regeneration in spinach (*Spinacia oleracea* L.) from callus on a gibberellin-free medium; however, the inclusion of 3 μM GA₃ stimulated adventitious shoot formation. In our study, optimal and minimal GA₃ concentrations suitable for spinach shoot regeneration were determined.

Seeds of 'High Pack' spinach (Alf Christianson Seed Co., Mount Vernon, Wash.) were grown under a 10-h photoperiod (350 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and a day/night cycle of 20C/16C. Fully expanded leaves were surface-sterilized according to Al-Khayri et al. (1991a).

One leaf disk, 5 mm in diameter, was cultured per 16 $\times$ 100-mm culture tube containing 5 ml medium. The medium consisted of inorganic salts of Murashige and Skoog (MS) (1962) supplemented with (in $\text{mg}\cdot\text{liter}^{-1}$) 100 *myo*-inositol, 0.4 thiamine-HCl, 0.5 nicotinic acid, 0.5 pyridoxine-HCl, and 30 g sucrose/liter. To induce callus, the medium was supplemented with 9.3 μM 6-furfurylaminopurine (kinetin) and 2.3 μM 2,4-dichlorophenoxyacetic acid (2,4-D). The medium was adjusted to pH 5.8 and solidified with 0.8% (w/v) tissue culture agar (Sigma, St. Louis). After autoclaving at 121C and 103 kPa for 15 min, the medium was cooled to 60C and enriched with 15% (v/v) filter-sterilized coconut water. The cultures were maintained in the dark at 20 $\pm$ 3C for 4 weeks.

Twenty calli per regeneration treatment were transferred to 25 $\times$ 150-mm tubes containing 15 ml medium. The regeneration media differed from the callus induction medium only in the content of growth regulators and contained 9.3 μM kinetin, 0.05 μM 2,4-D, and 0, 0.3, 0.7, 1.4, 2.9, or 7.2 μM GA₃. Growth regulators were added to the medium before autoclaving. The cultures were grown

with a 10-h photoperiod at 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and at 20 $\pm$ 3C.

The onset and the frequency of shoot formation from callus depended on the concentration of GA₃ in the regeneration medium (Fig. 1A). No regeneration on GA₃-free medium was observed for the duration of the experiment, 17 weeks. Shoot regeneration began within 4 weeks; after 5 weeks, the highest percentage of shoot formation (70%) was observed with the highest level of GA₃ (7.2 μM). By week 8, regeneration on 0.7, 1.4, and 7.2 μM GA₃ reached maximum frequency, but calli cultured at the other levels continued to increase in the number producing shoots. There was no significant increase in percentage shoot regeneration after week 8, and by week 11 regeneration ceased.

Shoot length increased with GA₃ concentration up to 1.4 μM but declined when GA₃ increased above this level (Fig. 1B). The stimulation of the growth of regenerated spinach shoot with lower levels of GA₃ and its inhibition by higher levels were similar to the pattern observed by Firman (1984) with potato (*Solanum tuberosum* L. cv. King Edward), where shoot length and multiplication rate increased up to five fold with 0.03 μM GA₃ but was strongly inhibited at higher concentrations. A stimulatory effect of GA₃ on regeneration was also observed by Economou and Spanoudaki (1986) in a study with *Gardenia jasminoides* Ellis. The inclusion of 0.5, 2.5, and 12.5 μM GA₃ in the medium increased the number and length of shoots produced.

To achieve maximum benefit from the stimulatory effect of GA₃ on spinach regeneration, calli should be cultured on a regeneration medium containing 7.2 μM GA₃ and the shoot-regenerating calli subcultured onto regeneration medium containing 1.4 μM GA₃ for shoot multiplication. This method would allow for maximum regeneration frequency and maximum shoot growth.

Literature Cited

- Al-Khayri, J.M., F.H. Huang, and T.E. Morelock. 1991a. Regeneration of spinach from leaf callus. *HortScience* 26:913-914.
Al-Khayri, J.M., F.H. Huang, T.E. Morelock, and T.A. Busharar. 1991b. Genotype-dependent response of spinach callus induction and shoot regeneration. *Plant Sci.* 78:121-127.
Appelgren, M. and O.M. Heide. 1972. Regeneration in *Streptocarpus* leaf discs and its regulation by temperature and growth substances. *Physiol. Plant.* 27:417-423.
Economou, A.S. and M.J. Spanoudaki. 1986. The influence of cytokinins and gibberellic acid on gardenia tissue cultures. *Scientia Hort.* 29:155-161.

Fig. 1. Effect of GA₃ concentration on shoot regeneration in spinach. (A) Cumulative percentage of calli-formed shoots by 5, 8, and 11 weeks on regeneration medium; (B) length of regenerated shoots as a function of GA₃ concentration after 11 weeks.

- Firman, D.M. 1984. Gibberellic acid as a media additive for in vitro propagation of potato (*Solanum tuberosum* L.). *J. Agr. Sci.* 103:703-704.
Murashige, T. 1964. Analysis of the inhibition of organ formation in tobacco tissue culture by gibberellin. *Physiol. Plant.* 17:636-643.
Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.
Pierik, R.L.M. 1987. In vitro culture of higher plants. Martinus Nijhoff Publishers, Boston. p. 71.
Pierik, R.L.M. and H.H.M. Steegmans. 1975. Analysis of adventitious root formation in isolated stem explants of *Rhododendron*. *Scientia Hort.* 3:1-20.
Ringe, F. and J.P. Nitsch. 1968. Conditions leading to flower formation on excised *Begonia* fragments cultured in vitro. *Plant Cell Physiol.* 9:639-652.
Schraudolf, H. and J. Reinert. 1959. Interaction of plant growth regulators in regeneration processes. *Nature* 184:465-466.

Received for publication 9 Dec. 1991. Accepted for publication 20 Apr. 1992. Published with approval of the Director of Arkansas Agricultural Experiment Station. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.