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# Photoperiod, Temperature, Gibberellin, and an Anti-gibberellin Affect Tuberization of Potato Stem Segments In Vitro

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***Abstract.*** **'Katahdin' potato plants were grown under conditions that did not induce tuberization (noninducing conditions) and the foliage was sprayed with either a growth retardant (BAS-111) at 1000 mg·L$^{-1}$ or distilled water. Other plants, grown under tuber-inducing conditions, received a foliar spray of gibberellic acid ($GA_3$) at 100 mg·L$^{-1}$ or distilled water. After 1 week, treatments were repeated. Two-node stem segments were excised from the apical, subapical, medial, and basal sections of each plant 72 hours after the second foliar treatment, disinfested, and inserted into flasks containing 50 mL of Murashige and Skoog medium (2% sucrose). After 3 weeks in a darkened incubator adjusted to 24 °C, tuberization response was evaluated. Orthogonal contrasts revealed significant differences between induced and noninduced controls for tuber number, diameter, and fresh mass. BAS-111 reduced rhizome length and increased tuber number, diameter, and fresh mass. $GA_3$ increased rhizome length, but reduced tuber number, diameter, and fresh mass. Node location influenced tuber development, as basal explants produced significantly more and larger tubers, as well as longer rhizomes, than did apical explants, and subapical segments produced more and larger tubers than did apical segments. There were no significant differences between medial and basal nodal segments with respect to tuber number or tuber/rhizome size. Chemical names used: 1-phenoxy-5,5-dimethyl-3-(1,2,4-triazol-1-yl)-hexan-5-ol (BAS-111); 2,4a,7-trihydroxy-1-methyl-8-methylenegibb-3-ene-1,10-carboxylic acid 1->4 lactone ($GA_3$).**

The tuber-forming sequence in *Solanum* species normally consists of rhizome development followed by tuberization of the subapical region of the rhizome (Booth, 1963). These processes are controlled by environmental factors, primarily temperature and photoperiod. Short days with cool night temperatures (inducing conditions) favor tuberization, while long days with high temperatures (noninducing conditions) delay or may inhibit the process entirely (Gregory, 1956; Slater, 1968).

Although several endogenous growth substances are reportedly involved in tuberization, some researchers (Dostal, 1945; Madec, 1963) suggested that a tuberizing hormone is synthesized or activated in potato leaves under inducing conditions and translocated to the rhizome region where tuber initiation occurs. Smith and Palmer (1970) demonstrated a cytokinin requirement for in vitro tuberization. Subsequent studies in this laboratory (Langille and Forsline, 1974; Mauk and Langille, 1978) support the theory that the tuberizing factor is a cytokinin. More recent findings (Koda et al., 1988, 1991) have implicated compounds related to jasmonic acid as having a causative role in tuberization.

Gibberellins reportedly have an inhibitory role in the tuberization process (Kumar and Wareing, 1974; Railton and Wareing, 1973). Okazawa (1959, 1960) observed that endogenous gibberellin content was highest in potato plants grown under noninducing conditions and decreased when plants were moved to inducing conditions. Furthermore, Hammes and Nel (1975) proposed that tuber formation is controlled by a balance between endogenous gibberellins and the tuberizing stimulus. They theorized that gibberellins must decrease below a minimum value for tuberization to occur. Recent studies (Langille and Hepler, 1992) demonstrated that basal leaf-bud cuttings from 'Katahdin' potato plants grown under noninducing conditions tuberized significantly better than did apical and subapical cuttings located in close proximity to the site of gibberellin synthesis.

Accordingly, this study was initiated to determine the effects of inductive conditions, an inhibitor of gibberellin synthesis and explant stem location on in vitro tuberization of 'Katahdin' nodal stem segments.

## Materials and Methods

*Plant material.* Etiolated sprouts were obtained by placing tubers of *Solanum tuberosum* L. cv. Katahdin in flats covered with moist perlite and paper, and incubating in a growth chamber adjusted to 28 °C as described by Forsline and Langille (1976). When sprouts had reached lengths of ≈9 cm, they were removed from the seed pieces, selected for uniformity, and planted in plastic pots (1350 mL) containing moistened Pro-Mix BX (Premier Brands, Stamford, Conn.). Forty-eight transplanted sprouts were then placed in a chamber under noninducing conditions [30 °C day/28 °C night, with a 16-h photoperiod providing a photosynthetic photon flux (*PPF*) of 140 μmol·m$^{-2}$·s$^{-1}$]. Each pot received 200 mL of 15N–30P–15K soluble fertilizer (Stern's Miracle-Gro; Port Washington, N.Y.). After 30 d, when plants had achieved heights of 55 to 60 cm, 24 plants were randomly selected and placed in a second chamber providing inducing conditions (21 °C day/13 °C night, with a 10-h photoperiod and the same *PPF*). The remaining plants were maintained under noninducing conditions. Following 48 h of conditioning in the chambers, the foliage of noninduced plants was sprayed to runoff with a hand atomizer with either distilled water or an aqueous solution of BAS-111 (BASF Corp., Research Triangle Park, N.C.) at 1000 mg·L$^{-1}$. Test solutions were allowed to air-dry on leaves before plants were returned to the noninducing chamber. Plants from the inducing chamber were sprayed with distilled water or with 100 mg·L$^{-1}$ $GA_3$ (Sigma Chemical, St. Louis, Mo.) to foliar runoff. All test solutions contained Tween 80 surfactant (Fisher Scientific, Pittsburgh) at the rate of 1 mg·L$^{-1}$. A second application was made 1 week later. Seventy-two hours after the final treatment, the plants were removed from the chambers and the central axis of each was divided into apical, subapical, medial, and basal two-node stem segments, according to Ewing and Wareing (1978).

Nodal stem segments were identified by treatment/location, placed in separate cheesecloth bags, and disinfested using a Clorox®, ethanol, sterile water sequence, as described by Forsline and Langille (1976). Ends of segments were then trimmed and their lengths adjusted to ≈4 cm. These latter procedures were conducted in a laminar flow hood.

*In vitro culture.* Basal ends of each segment were inserted into 125-mL Erlenmeyer flasks containing 50 mL of sterile MS medium (Murashige and Skoog, 1962), containing 2% sucrose, 100 mg·L$^{-1}$ inositol, 5 mg·L$^{-1}$ thia-

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mine-HCl, 2 mg·L$^{-1}$ Ca-pantothenate, 0.8% agar, and adjusted to pH 5.6. Flasks were stoppered with cotton plugs, sealed with aluminum foil, and placed in an incubator in the dark at 24 °C. Each treatment was replicated five times. After 3 weeks in culture, buds were evaluated for rhizome and/or tuber development. Data from three experiments were combined and analyzed as a completely random design. Orthogonal contrasts were used to test significance of observed differences between means.

Table 1. Effect of induction conditions and growth regulator treatments on contrasts of mean responses of potato nodal stem segments cultured in vitro.[z] Observations made after 3 weeks in culture.

| Induction | Foliar treatment | Rhizome length (cm) | Tuber no. | Tuber diam (cm) | Tuber fresh mass (mg) |
|---|---|---|---|---|---|
| Yes | Water | 3.7 | 1.2 | 0.40 | 345.9 |
| | | * | ** | ** | ** |
| | $GA_3$ | 4.5 | 0.1 | 0.03 | 4.1 |
| | | NS | ** | * | ** |
| --- | Water | 4.1 | 0.2 | 0.15 | 55.4 |
| | | ** | ** | ** | * |
| No | BAS-111 | 2.6 | 0.7 | 0.31 | 239.7 |

[z]Specific orthogonal contrasts indicated by brackets.
[NS, *, **]Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

Table 2. Effect of location on the donor plant from which explants were taken on selected contrasts of mean responses of nodal stem segments cultured in vitro.[z] Observations made after 3 weeks in culture.

| Stem location | Rhizome length (cm) | Tuber no. | Tuber diam (cm) | Tuber fresh mass (mg) |
|---|---|---|---|---|
| Apical/subapical | 2.4 | 0.3 | 0.14 | 37.2 |
| vs. | ** | ** | ** | ** |
| Medial/basal | 5.1 | 0.8 | 0.35 | 285.4 |
| Apical | 1.8 | 0.1 | 0.05 | 3.2 |
| vs. | ** | ** | ** | NS |
| Subapical | 3.0 | 0.5 | 0.23 | 71.2 |
| Medial | 4.8 | 0.8 | 0.36 | 284.1 |
| vs. | NS | NS | NS | NS |
| Basal | 5.4 | 0.9 | 0.34 | 286.7 |

[z]Specific orthogonal contrasts indicated by brackets.
[NS, *, **]Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

## Results

Mean rhizome length on induced control explants did not differ from that on noninduced controls. However, induction increased tuber number, tuber diameter, and fresh mass (Table 1). Treatment with $GA_3$ significantly increased rhizome length of induced explants (Table 1). Conversely, foliar application of BAS-111 to plants growing under noninducing conditions significantly reduced rhizome length of explants. Treatment of induced plants with $GA_3$ significantly reduced tuber number, diameter, and fresh mass on explants, whereas treatment of noninduced plants with BAS-111 had the opposite effects.

Position on the stem had a significant effect on the response observed (Table 2). For example, rhizome length and tuber number, diameter, and fresh mass were significantly greater for lower explants (medial/basal) than for the upper two explants (apical/subapical). Contrasts revealed no significant differences between medial and basal locations for any characteristics (Table 2). When apical and subapical locations were compared, however, they differed significantly in rhizome length, tuber number, and tuber diameter, but tuber fresh mass was not influenced.

## Discussion

Consistent with studies by Gregory (1956) and Forsline and Langille (1976), prior exposure of donor plants to inducing conditions enhanced tuberization on cultured control segments (Table 1). Hammes and Nel (1975) suggested that tuberization is controlled by a balance of endogenous gibberellins and a tuber-forming stimulus, with gibberellins acting as antagonists to in vitro tuberization (Palmer and Barker, 1972). Several studies (Lovell and Booth, 1967; Tizio, 1971) have demonstrated a similarly inhibitory effect on tuber formation when GA was applied to potato plants. In the present study, foliar application of $GA_3$ to induced plants also adversely affected tuber formation, as evidenced by reduced tuber number, diameter, and fresh mass (Table 1). Since rhizomes are more plentiful and longer when potato plants are grown under long days (Chapman, 1958), conditions associated with elevated endogenous gibberellins (Okazawa, 1960), the $GA_3$ treatment could be expected to increase rhizome length on induced explants.

Since the inhibitory role of gibberellins in the tuber-forming process(es) has been documented previously, a triazole growth retardant, such as BAS-111, which functions by blocking oxidative reactions leading to gibberellin synthesis (Rademacher et al., 1984), might be expected to increase tuber yield of explants following application to donor plants growing under noninducing conditions. The BAS-111 treatment achieved the desired result. In keeping with Hammes and Nel's (1975) theory that endogenous gibberellins must fall below a minimum for tuber initiation to occur, one can speculate that BAS-111 reduced gibberellin synthesis in plants growing under conditions that normally favor gibberellin production. The more than 3-fold increase in tuber number and yield associated with the BAS-111 treatment supports this hypothesis. In a related study, tuberization was increased nearly 3-fold in stem cuttings when noninduced donor plants were treated with BAS-111 (Langille and Hepler, 1992).

If gibberellins are synthesized in immature apical leaves as proposed by Jones and Phillips (1966), tuber formation in explants should increase with distance from the apex. This proved to be the case; tuber number, diameter, and fresh mass for the medial and basal segments combined were all significantly greater than the combined response for segments taken from the upper portion of the plant (Table 2). This trend also held for tuber number and diameter when apical and subapical locations were compared; however, medial and basal segments did not differ in their ability to produce tubers. Chapman (1958) observed that basal segments of induced plants tuberized more readily than did apical segments. An earlier study conducted in this laboratory (Forsline and Langille, 1976), demonstrated that apical segments tuberized significantly better than those from medial or basal locations. Note, however, that the 6% sucrose concentration used in culture media for this earlier study may have masked the effect of position on the stem. Mes and Menge (1954) noted that elevated sucrose concentrations stimulated tuberization in cultured potato stem segments. Based on the data of Jones and Phillips (1966) it was expected that rhizome growth would be greatest on those segments closest to the stem apex. This was not the case; average length was greater for basal than for apical segments, although medial and basal segments did not differ in the length of rhizomes produced. These findings agree with those of Booth (1963), who observed that the first formed and longest rhizomes developed at the lowest nodes of *Solanum andigena* Hawkes.

Results of the present study support the theory advanced by Hammes and Nel (1975) that endogenous gibberellins, in concert with a yet to be identified "tuberizing factor," control tuber formation in the potato. Several compounds have been advanced as candidates for this factor (Koda et al., 1988; Mauk and Langille, 1978), but its identity remains unknown. Inhibitors of gibberellin synthesis could have a place in the management protocols used in commercial potato production, especially in northern locations where yield may be decreased by short growing seasons. Additional field experimentation will be required to determine their effectiveness.

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