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Comparative Effects of Gibberellic Acid and N^6-Benzyladenine on Dry Matter Partitioning and Osmotic and Water Potentials in Seedling Organs of Dwarf Watermelon*

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Abstract. Apical applications of 0.2 μg N^6-benzyladenine (BA), a synthetic cytokinin, or 5 μg of gibberellic acid (GA_3) significantly enhanced hypocotyl elongation in intact dwarf watermelon seedlings over a 48-h period. Accompanying the increase in hypocotyl length was marked expansion of cotyledons in BA-treated seedlings and inhibition of root growth by both compounds. A study on dry matter partitioning indicated that both growth regulators caused a preferential accumulation of dry matter in hypocotyls at the expense of the roots; however, GA_3 elicited a more rapid and greater change than did BA. In comparison to untreated seedlings, BA decreased total translocation of metabolites out of the cotyledons. Water potentials of cotyledons and hypocotyls were determined by allowing organs to equilibrate for 2 h in serial concentrations of polyethylene glycol 4000. Osmotic potentials were determined by thermocouple psychrometry. During periods of rapid growth in cotyledons and hypocotyls of BA-treated seedlings and in hypocotyls of GA-treated seedlings, the osmotic potential increased and the turgor pressure decreased in relation to untreated seedlings, indicating that cell wall extensibility was being increased. Osmotic potentials were lower in hypocotyls of GA-treated than in those of BA-treated seedlings, even though growth rates were higher in GA-treated seedlings, indicating that the latter treatment was generating more osmotically active solutes in hypocotyls.

Application of either N^6-benzyladenine (BA) or gibberellic acid (GA_3) promotes hypocotyl elongation in dwarf watermelon seedlings (Loy 1980, Loy and

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Liu 1974). BA-enhancement of hypocotyl elongation is primarily a result of increased cell elongation, whereas GA_3 markedly increases both cell length and cell number. Both growth regulators inhibit root growth in seedlings, and GA promotes expansion of intact (Loy 1980) or excised (Longo et al. 1978) cotyledons. Enhancement of hypocotyl elongation in dwarf watermelon by both cytokinins and gibberellins appears unique and permits a comparative study of the effects of these two classes of growth regulators on growth, solute potential, and water potential in an intact organ.

Cell enlargement requires a stretching of the existing cell wall plus synthesis of new wall material to maintain the properties of the cell wall for continued extension (Rayle et al. 1970). The driving force for cell elongation is turgor pressure that exceeds some yielding threshold of the cell wall (Green et al. 1971). The rate of water movement into a cell, and thus cell enlargement, can be accelerated by wall loosening together with sufficient cell osmolality for maintaining some minimum turgor pressure. Since both BA and GA_3 increase cell elongation in dwarf watermelon, it is of interest to know if they affect this process through similar mechanisms.

GA_3 increases wall extensibility in *Avena* stem segments (Adams et al. 1975), pea stem segments (Lockhart 1960), and lettuce hypocotyl sections (Stuart and Jones 1977), but not in cucumber hypocotyls (Cleland et al. 1968, Katsumi and Kazama 1978). Cytokinin-induced growth of radish cotyledons was accompanied by increased production of osmotically active reducing sugars (Huff and Ross 1975, Bewli and Witham 1976) and also by increased cell wall extensibility (Thomas et al. 1981). Longo et al. (1978) reported that BA promoted production of reducing sugars in excised watermelon cotyledons. In addition, they concluded that BA increased wall extensibility because the osmotic potential of the more rapidly expanding BA-treated cotyledons was always less negative than that of control cotyledons.

In the present study, we compared the effects of GA_3 and BA on growth by determining solute and water potentials of hypocotyls and cotyledons, and dry matter partitioning in seedling organs of dwarf watermelon.

Materials and Methods

Plant Material

Seedlings of a dwarf (dw-2) inbred strain of watermelon (*Citrullus lanatus* [Thunb.] Matsu & Nakai), designated WB-2, were grown on absorbent wadding in Petri dishes under continuous fluorescent light at 30°C as described previously (Loy 1980).

Hormone Treatments

Seedlings were selected for hypocotyl uniformity and treated at 120 h. Either 0.2 μg of BA (Sigma) or 5 μg of GA_3 (Sigma) in a 10-μl aqueous droplet was administered with a microsyringe to the apex of each seedling, between the cotyledons. These dosages elicited near maximum hypocotyl elongation.

Fresh and Dry Weight Determinations

Fresh and dry weight measurements of cotyledons, hypocotyls, and roots were taken at 6-h intervals for the first 48 h following treatment. Because of the large number of seedlings involved, GA_3 and BA experiments were conducted at separate times, each with its own set of control seedlings.

Water and Osmotic Potential Determinations

Water and osmotic potentials of cotyledons and hypocotyls were determined at 0, 12, 24, and 48 h following treatment. Two organs per replication and five replications were used for each determination for a given experiment. The cotyledons and hypocotyls were excised from intact seedlings at the time of measurement. Water potentials were determined by placing the cotyledons or hypocotyls in 60-mm covered Petri dishes in serial concentrations of polyethylene glycol 4000 (PEG) of known osmotic potential. Osmotic potentials were determined in each experiment by thermocouple psychrometry, using a Wescor HR-33T dew-point microvoltmeter that was calibrated with NaCl solutions. The osmotic potential of the solution that did not induce a measurable change in fresh weight of the tissue after 2 h was assumed to equal the water potential of the organ. This was estimated from a regression line fitted to several data points for each experiment. Osmotic potentials were determined by saturating filter paper discs with cell sap from homogenized cotyledons or hypocotyls. Discs were immediately placed in a Wescor C-52 chamber connected to the Wescor dew-point microvoltmeter.

Results

Seedling Development

Both GA_3 and BA treatments increased hypocotyl fresh weight and inhibited root growth of intact seedlings (Fig. 1A, B, C). BA markedly promoted cotyledon expansion within 6 h following treatment. GA_3 induced a slight fresh weight increase in cotyledons during the first 6 h following treatment, but elicited no further changes in fresh weight over the 48-h treatment period. Cotyledon fresh weight of untreated seedlings remained nearly constant during the 48-h measurement period.

Dry Matter Partitioning

The radiant flux density of 4.2 $\mu Em^{-2}s^{-1}$(400–700 nm) under which the seedlings were grown should have been below the light compensation point. Nonetheless, significant decreases in total seedling dry matter due to respiration were not detected over the 48-h treatment period among any of the treatments.

Dry weights of cotyledons decreased over time for all treatments (Fig. 2A). However, there was a smaller decrease in cotyledon dry weight in BA-treated seedlings than in untreated or GA_3-treated seedlings. Both BA and GA_3 caused

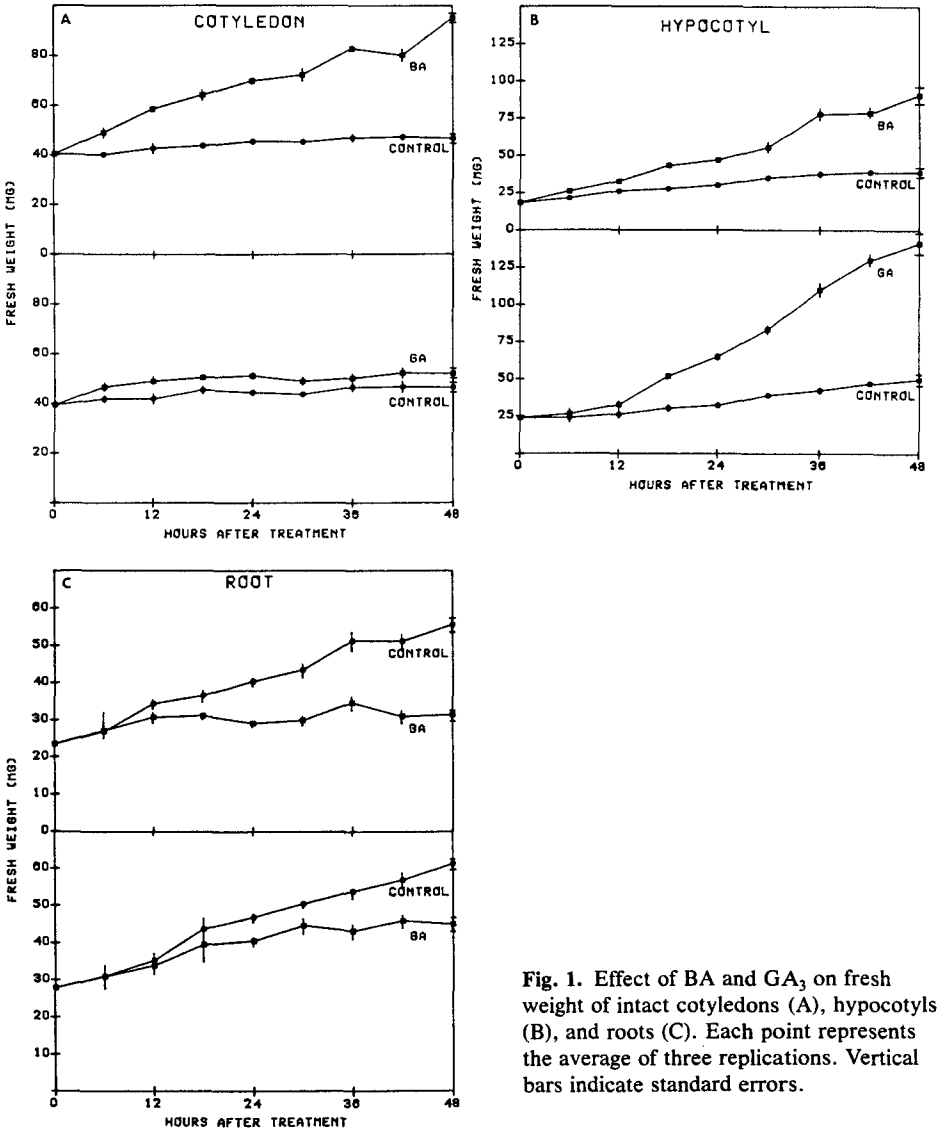

Fig. 1. Effect of BA and GA_3 on fresh weight of intact cotyledons (A), hypocotyls (B), and roots (C). Each point represents the average of three replications. Vertical bars indicate standard errors.

a preferential translocation of metabolites to hypocotyls at the expense of the roots (Fig. 2B, C). Maximum accumulation of dry matter in hypocotyls was obtained with GA_3 treatment. The GA_3-promoted increase in hypocotyl dry weight was detected by 12 h. A significant promotive effect of BA on hypocotyl dry weight was not detectable until 30 to 36 h following treatment.

Water and Osmotic Potentials of Intact Cotyledons

The effect of BA on water and osmotic potentials of intact cotyledons is illustrated in Fig. 3A. The water potential of control cotyledons increased slightly

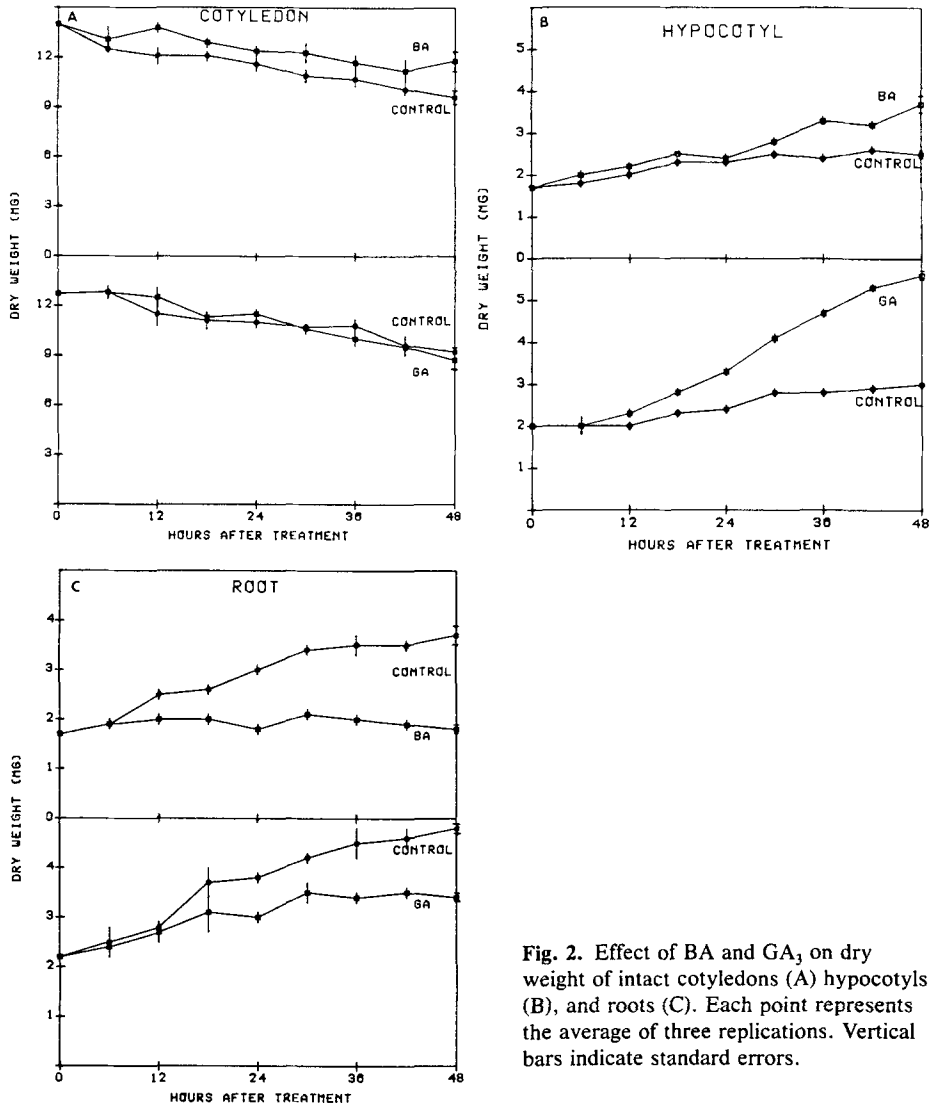

Fig. 2. Effect of BA and GA_3 on dry weight of intact cotyledons (A) hypocotyls (B), and roots (C). Each point represents the average of three replications. Vertical bars indicate standard errors.

from 12 to 24 h and then remained constant for the next 24 h. The osmotic potential continuously increased over 48 h with the most rapid increase between 0 and 24 h. The water potential of BA-treated cotyledons did not differ statistically from controls except at 12 h following treatment, when it was less negative. The osmotic potential of BA-treated cotyledons was always less negative than in control cotyledons, and the magnitude of the difference was greatest within 12 h following treatment.

Water potentials of GA_3-treated cotyledons were significantly more negative than control cotyledons at 24 h, but did not differ significantly at 12 and 48 h (Fig. 3B). At no time did the osmotic potential of GA_3-treated cotyledons differ

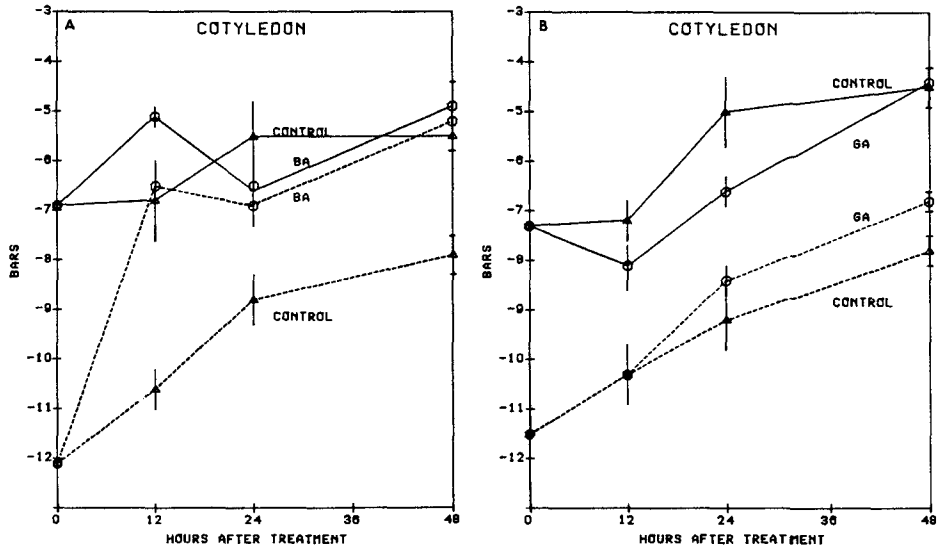

Fig. 3. Effect of BA (A) and GA_3 (B) on water potential (———) and osmotic potential (– – – – –) in intact cotyledons. Each point represents the average of five replications. Vertical bars indicate standard errors.

significantly from that of control seedlings, although values tended to be less negative at 24 and 48 h.

Water and Osmotic Potentials of Intact Hypocotyls

Water potentials of hypocotyls in untreated seedlings remained constant over time while the osmotic potential increased gradually over 48 h (Fig. 4A, B). BA had no significant effect on hypocotyl water potentials; however, the mean values obtained were always less negative in BA-treated seedlings than in untreated seedlings (Fig. 4A). Within 12 h following BA treatment, there was a sharp increase in the osmotic potential, indicating that the concentration of solutes was not being maintained during BA-promoted growth. By 48 h, turgor pressure (calculated from differences between water potentials and osmotic potentials) of BA-treated hypocotyls was approaching 0 bars (Table 1).

The osmotic potential of GA_3-treated hypocotyls increased significantly more than that of control hypocotyls between 12 and 48 h, but there was a corresponding increase in water potential between 24 to 48 h so that turgor pressure was maintained during growth over that period.

Discussion

In hypocotyls of dwarf watermelon seedlings, both BA and GA_3 treatments markedly increased osmotic potentials (Fig. 4) and decreased estimated turgor pressures (Table 1) while promoting elongation, indicating that longitudinal cell wall extensibility was being enhanced. Likewise, BA-induced cotyledon ex-

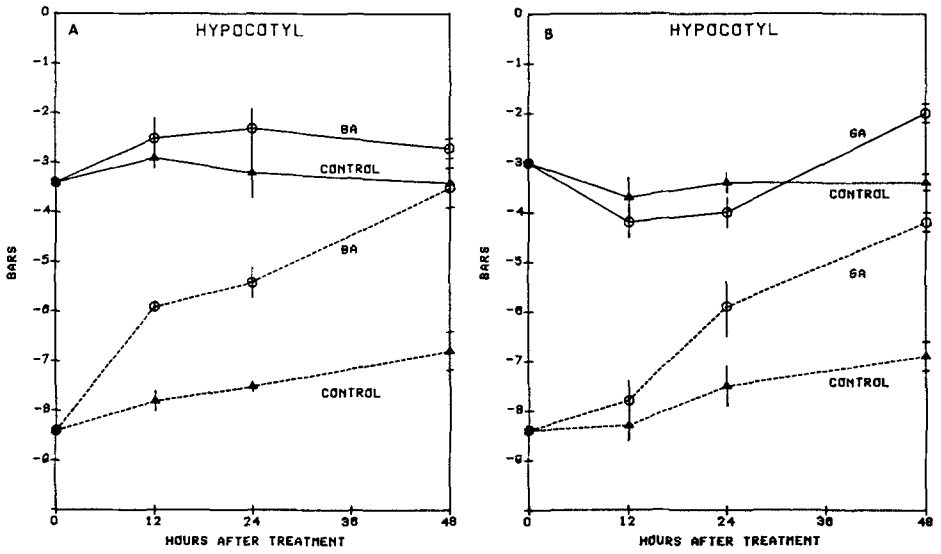

Fig. 4. Effect of BA (A) and GA_3 (B) on water potential (———) and osmotic potential (– – – –) in intact hypocotyls. Each point represents the average of five replications. Vertical bars indicate standard errors.

pansion was accompanied by a sharp increase in osmotic potential (Fig. 3A) and a decrease in estimated turgor (Table 1). Growth enhancement concomitant with appreciably decreased turgor clearly implicates cell wall loosening as being the primary factor mediating BA-induced growth of cotyledons. The results thus corroborate previous work demonstrating that an increase in cell wall extensibility is a major component of tissue expansion induced by BA (Longo et al. 1978, Thomas et al. 1981, Rayle et al. 1982) and GA_3 (Adams et al. 1975, Lockhart 1960, Stuart and Jones 1977). In BA-treated seedlings, solutes may have been growth-limiting in cotyledons between 24 to 48 h and in hypocotyls at 48 h, as suggested from the low estimated turgor pressures between 0 and 1 bar (Table 1). These turgor values may be too low, as discussed below; however, low turgor in these organs was suggested by their flaccid state.

Because mixed cell sap was used for determining osmotic potentials by a psychrometric method in the present study, cell wall water could have diluted cell sap, resulting in slightly less negative estimates of osmotic potentials (Wenkert 1980). Such errors, however, should not detract from the conclusions drawn from the large differences in osmotic potentials between treated and untreated seedlings.

Determination of water potentials in seedling organs according to changes in volume of excised tissues in serial concentrations of an osmoticum is subject to numerous small errors, such as solute leakage from tissue, effect of excision on growth, and difficulty in detecting growth changes in small tissues. More recently, Rayle et al. (1982) have reported a problem of osmotic adjustment exhibited by excised cucumber cotyledons floated in PEG-4000 solutions for different time periods. Incubation periods of 1 to 2 h were considered most

Table 1. Estimated turgor pressure values of intact cotyledons and hypocotyls from seedlings treated apically with 0.2 μg BA or 5 μg GA.

Hours following treatment	Treatment	Cotyledons (bars)[a]	Hypocotyl (bars)[a]
0	Control	4.6 ± 0.4	5.0 ± 0.3
12	Control	3.5 ± 0.4	4.8 ± 0.1
	GA	2.2 ± 0.4	3.6 ± 0.4
	BA	1.4 ± 0.3	3.4 ± 0.4
24	Control	4.0 ± 0.4	4.2 ± 0.3
	GA	2.1 ± 0.2	1.9 ± 0.2
	BA	0.8 ± 0.4	3.2 ± 0.5
48	Control	3.1 ± 0.4	3.6 ± 0.3
	GA	2.5 ± 0.2	2.2 ± 0.2
	BA	0.3 ± 0.5	0.8 ± 0.5

[a] Estimated turgor pressures were calculated from the difference between the water potential and osmotic potential, ignoring the effects of matrix potentials. Each value represents the average of ten replications for controls and five replications for GA and BA ± standard errors.

reliable for estimating ψ potentials. In our experiments, watermelon tissues were incubated in PEG solutions for 2-h periods, so that errors due to osmoregulation should have been small, resulting in slightly more negative water potentials.

Despite the difficulties in accurately measuring osmotic and water potentials, major repeatable changes in osmotic and water potentials were observed over time and among treatments in seedling organs of watermelon. Thus, we believe our measurements provide a reasonable basis for relating the components of water potential to growth of seedling organs.

Over short time periods, the watermelon seedlings used in this study can be considered as a closed system because the effects of photosynthesis and respiration on changes in total dry matter are negligible. Thus, the observed changes in distribution of dry matter among seedling organs over time indicate changes in patterns of translocation of assimilates and probably in mobilization of stored reserves. In comparison to BA, GA_3 elicited a considerably larger and much faster increase in dry matter accumulation in hypocotyls (Fig. 2B), thus providing additional assimilates for cell wall synthesis and maintenance of solute levels during GA_3-enhanced growth. The amounts of solutes, principally potassium and reducing sugars, are markedly increased in hypocotyls by GA_3 treatment (Zack 1981). The GA_3-mediated increase in dry matter in hypocotyls was not the result of increased translocation out of the cotyledons, but rather was the result of decreased accumulation of dry matter in the roots. BA also inhibited dry matter accumulation in roots, but its application resulted in a much smaller dry matter increase in hypocotyls because of suppression of assimilate translocation out of cotyledons (Fig. 2).

In conclusion, it appears that GA_3 and BA not only affect cell wall extensibility in hypocotyls of dwarf watermelon seedlings, but also affect patterns of assimilate distribution and probably mobilization of cotyledon reserves.

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