Effects of Selected Putative Inhibitors of Ribonucleic Acid or Protein Synthesis on Adventitious Root\textsuperscript{1} Formation in Mung Bean Cuttings

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Abstract. Selected putative inhibitors of ribonucleic acid (RNA) synthesis (actinomycin D and 6-methylpurine) or protein synthesis (cycloheximide and puromycin) were examined for their effects on root formation in mung bean (Vigna radiata (L.) R. Wilcz.) cuttings in the presence or absence of naphthaleneacetic acid (NAA). Only 6-methylpurine completely inhibited root formation at concentrations that did not cause visible injury. Cycloheximide was most inhibitory when applied at the same time as NAA. Application of 6-methylpurine up to 12 hours after NAA uptake completely blocked root formation; thereafter its effect declined with time. This decline in response was correlated with enlargement of the nucleus and nucleolus in hypocotyl cells preparatory to cell division.

The process of root formation involves both cell division and enlargement, and therefore is dependent on synthesis of nucleic acids and proteins. Molnar and La Croix (19) showed that protein synthesis preceded deoxyribonucleic acid (DNA) synthesis and cell division in preformed root initials of Hydrangea macrophylla ‘Kunhe’. Extensive changes in enzymatic activity were demonstrated by histochemical staining of the cells responsible for root initiation (20).

Because the process of root formation is dependent upon the synthesis of nucleic acids and proteins, chemicals which interfere with or block nucleic acid and/or protein synthesis should inhibit rooting. Actinomycin D, 2,6-diaminopurine, and 2-thiouracil, which inhibit nucleic acid synthesis, also inhibit rooting (8,9,11,18,22). However, Mitsuhashi et al. (18) reported stimulation of rooting in Azukia (Azukia angularis) cuttings by actinomycin D depending on the time of application of the inhibitor. Cycloheximide and puromycin inhibit both protein synthesis and rooting (11,14,18). Mitsuhashi et al. (18) found puromycin inhibited rooting of Azukia cuttings only when applied 24 hr after preparation of cuttings. A third inhibitor of protein synthesis, chlorophenicol, may (8,14) or may not (18) inhibit rooting depending upon species.

This investigation was undertaken to determine the effects of the RNA synthesis inhibitors actinomycin D (7,10) and 6-methylpurine (12,13), and the protein synthesis inhibitors cycloheximide (3,16) and puromycin (2,7,24) on adventitious root formation in mung bean cuttings.

Materials and Methods

Plant material and assay procedures. ‘Oriental Giant’ mung beans from one seed lot were used. Germination, growth, cutting preparation, and assay conditions were described by Blazich and Heuser (5). Three replications (vials) of 10 cuttings each were used per treatment, and roots were counted after 5 days.

Chemicals. Actinomycin D, cycloheximide, 6-methylpurine, and puromycin were purchased from Sigma Chemical Co., St. Louis, Mo., and naphthaleneacetic acid (NAA) from Nutritional Biochemicals Corp., Cleveland, Ohio.

Rooting response. Rooting response to the inhibitors in the presence or absence of 10^{-4} M NAA was determined at the following molar concentrations: actinomycin D (10^{-9} to 5 \times 10^{-6}), cycloheximide, 6-methylpurine, and puromycin (10^{-9} to 10^{-3}). Assays were conducted by placing 10 cuttings in a shell vial with 1 ml of test solution containing 5 ppm boric acid. Following the uptake of each test solution, cuttings were watered with autoclaved double-distilled water as described by Blazich and Heuser (5).

Time course studies. Cuttings were initially pretreated in shell vials (10 cuttings per vial) with 1 ml of 10^{-4} M NAA test solution containing 5 ppm boric acid. About 1.5 hr were required for absorption of the pretreating auxin solution. Timing for inhibitor treatments started after uptake of the NAA pretreating solution. At appropriate intervals from 0 to 48 hr after pretreatment, cuttings (10 per vial with 3 replicates) were transferred from vials containing autoclaved double-distilled water to vials containing either 1 ml of cycloheximide (3 \times 10^{-5} M), or 6-methylpurine (6 \times 10^{-5} M). After solution uptake, autoclaved double-distilled water was added to the vials.

Two sets of controls were used. One set consisted of cuttings initially treated with the inhibitor plus auxin (10^{-4} M NAA) containing 5 ppm boric acid. The other control consisted of cuttings treated with 10^{-4} M NAA containing 5 ppm boric acid. The number of roots on each cutting was determined after 5 days.

Results

Effects of RNA or protein synthesis inhibitors on rooting. Actinomycin D, 6-methylpurine, cycloheximide and puromycin all inhibited adventitious root formation (Fig. 1 and 2). Actinomycin D slightly inhibited rooting at 10^{-7} M (Fig. 1). Inhibition of rooting increased with increasing concentration, all cuttings being killed at 5 \times 10^{-6} M. All concentrations which inhibited rooting also caused observable injury in the form of necrotic areas on the...
leaves, epicotyl, and hypocotyl. Injury increased with increasing concentration. Solution uptake was retarded by $10^{-5}$M actinomycin D (Fig. 1).

Concentrations of $10^{-5}$M or higher 6-methylpurine also inhibited rooting (Fig. 1). Complete inhibition of root formation with no observable injury to the cuttings occurred at $6 \times 10^{-5}$ to $10^{-4}$M 6-methylpurine. At a concentration of $10^{-3}$M the cuttings were killed. No delay in solution uptake was observed.

Inhibition of rooting by cycloheximide was first observed at $1 \times 10^{-6}$M (Fig. 2), and increased with concentration, the cuttings being killed at $10^{-4}$M. Injury was evident at $2 \times 10^{-6}$ to $10^{-5}$M (Fig. 2). At concentrations of $2 \times 10^{-6}$ and $3 \times 10^{-6}$M injury took the form of small necrotic areas scattered on some leaves. At $4 \times 10^{-6}$ and $5 \times 10^{-6}$M necrotic areas occurred on all leaves and epicotyl and hypocotyl injury became noticeable on some cuttings. At $10^{-3}$M all cuttings were badly injured, with more necrosis on the leaves, epicotyl, and hypocotyl. The rate of test solution uptake was reduced at concentrations higher than $2 \times 10^{-6}$M.

Puromycin inhibited rooting at concentrations of $6 \times 10^{-4}$M and above (Fig. 2). All concentrations which inhibited rooting induced necrotic areas on the leaves. Puromycin had no effect on test solution uptake.

**Time course studies.** Cycloheximide was most inhibitory when applied simultaneously with auxin (Fig. 3). Cycloheximide treatment within 24 hr of pretreatment with auxin resulted in a fairly constant level of inhibition. However, this level of inhibition was less than when cycloheximide and auxin were added together (Fig. 3). Inhibition was not evident when cycloheximide was applied 48 hours after auxin pretreatment.

If 6-methylpurine was added at 0 to 12 hr, rooting was totally inhibited (Fig. 3). Inhibition of rooting decreased between 12 and 16 hr and was negligible at 48 hr.

**Discussion**

Several inhibitors of RNA or protein synthesis inhibit rooting. Actinomycin D inhibits root formation in pea (8) and willow (11), in the latter increasing with concentration. However, Mitsuhashi

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**Fig. 1.** Effect of actinomycin D and 6-methylpurine on adventitious root initiation in mung bean cuttings. Each point is the mean for 30 cuttings. The vertical line through each point represents ± 1.0 s.d.

**Fig. 2.** Effect of cycloheximide and puromycin on adventitious root initiation in mung bean cuttings. Each point is the mean for 30 cuttings. The vertical line through each point represents ± 1.0 s.d.
The vertical line through each point represents ±0.05 SD.

Puromycin inhibits rooting in maize seedlings (14) and Azukia cuttings (18) and our results show similar responses in mung bean.

Of the putative inhibitors of RNA or protein synthesis, only 6-methylpurine partially or completely blocked rooting at concentrations (6 × 10⁻³ to 10⁻⁴ M) which did not kill or injure the cuttings (Fig. 1), whereas actinomycin D, cycloheximide, and puromycin injured cuttings at concentrations which inhibited rooting. Thus inhibition of rooting by these 3 compounds may be a secondary effect due to injury. The specificity of action of cycloheximide has been questioned, as a wide range of deleterious effects produced have been reported (15,17). Many researchers have worked with actinomycin D, cycloheximide, puromycin, and related compounds with respect to rooting and none have reported injury to plant tissue. Injury may be related to tissue maturity.

Cycloheximide appeared to be active at more than one stage in the sequence of events leading to adventitious root formation. If cycloheximide was added at the same time as auxin, rooting was inhibited 75%, whereas rooting of cuttings treated first with auxin then with cycloheximide was inhibited only 25% (Fig. 3). The cycloheximide results indicate the possible existence of de novo protein synthesis specific for root initiation early in the process. However, the quick auxin response may not be related to protein synthesis but to some other mechanism of cycloheximide action (15,17).

The data presented for 6-methylpurine (Fig. 3) are similar to those of Chandra, et al. (6) for streptomycin inhibition of rooting in mung bean cuttings. Root formation was completely blocked when the cuttings were treated with streptomycin during the first 12 hr of incubation, whereas treatment 24 hr after the cuttings were placed in water was only moderately inhibitory; treatment at 48 hr was without effect (6).

Oppennooth (21) used actinomycin D to inhibit rooting of Phaseolus vulgaris L. leaf cuttings, which form adventitious roots on the petiole. His data parallel our data for 6-methylpurine inhibition (Fig. 3). He did not report any actinomycin D injury.

The decline in 6-methylpurine inhibition between 12 and 16 hr can be correlated with histological changes occurring in the hypocotyl. Blazich and Heuser (4) reported that 12 hr after absorption of the test solution by both control and NAA-treated mung bean cuttings, both the nucleus and nucleolus began to enlarge in a few of the cells having the potential for adventitious root formation. Such activity is characteristic of cells preparing for division. By 16 hr hypocotyl sections from both control and NAA-treated cuttings showed an increase in activity (4). Adamson (1) reported that tuber cells of Jerusalem artichoke (Helianthus tuberosus L.) which were preparing for cell division contained large, dense nuclei and nucleoli (1). RNA content in such nuclei was 3 to 4 times greater than in nuclei from cells which were not preparing for division.

Most RNA synthesis presumably occurs in the nucleus during transcription (23). Thus, the increased nuclear and nucleolar activity observed at 16 hr (14) may be interpreted as reflecting an increase in RNA synthesis during transcription. Since 6-methylpurine is a suspected inhibitor of RNA synthesis in the mung bean system, the loss of sensitivity to 6-methylpurine between 12 and 16 hr that the RNA synthesis and/or accumulation necessary for root initiation and development had occurred during this period.

**Literature Cited**

Temperature-stress-induced Production of Abscisic Acid and Dihydrophaseic Acid in Warm- and Cool-season Crops

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Abstract. Abscisic acid (ABA) metabolism of 6-week-old seedlings of cool- and warm-season crops was determined after a 24-hr exposure to supra- and sub-optimal temperatures. Temperatures were grown at 25°C and then exposed to 10, 25, or 40°C. After a 24-hr exposure, free (FABA) and hydrolyzable (HABA) abscisic acid and dihydrophaseic acid (DPA) were measured in the plant tops by gas chromatography. Warm-season crops, exposed to 10°C exhibited elevated levels of FABA, HABA and DPA compared to those plants exposed to 25 or 40°C. Among cool-season crops, only peas had higher FABA and HABA levels at 40°C than at 10 or 25°C, while beets had lower levels of HABA at 25°C than at 10 or 40°C. DPA existed at much higher concentrations than FABA and HABA in all plants. The increases in ABA and DPA in warm-season crops exposed to 10°C are attributed to low temperature stress.

Abscisic acid (ABA), a naturally occurring plant hormone, fills major roles in plant growth and development (1, 10, 11, 12) that include an association with stress (3, 4, 5, 7, 14, 15, 20, 25). The higher its ABA, the more resistance a plant will have to sub-zero temperatures (10, 17, 18). Several investigators believe that ABA can increase a plant’s tolerance to any sub-optimal condition (9, 10, 17).

One limitation affecting the production of a large group of economically important agricultural corps is the temperature under which the plants can grow, survive, and produce. In tomato plants exposed to low or high temperatures, the highest levels of ABA were observed under 10°C (2). These elevated ABA levels were attributed to the magnitude of the temperature stress on the plant, since tomato is a warm-season crop.

The objective of this study was to determine whether concentrations of ABA and its metabolites in a group of cool- and warm-season crops were altered by exposure to supra- and sub-optimal temperatures.

Materials and Methods

Warm-season crops: bean (Phaseolus vulgaris L. cv. Burpee Stringless Green Pod); corn (Zea mays L. cv. Golden Jubilee); muskmelon (Cucumis melo L. cv. Hales Best); eggplant (Solanum melongena L. cv. Ichiban); and okra (Hibiscus esculentus L. cv. Dwarf Green) and cool-season crops: beet (Beta vulgaris L. cv. Early Wonder); lettuce (Lactuca sativa L. cv. Great Lakes); cabbage (Brassica oleracea L. cv. Savoy); radish (Raphanus sativus L. cv. Scarlet Globe); and pea (Pisum sativum L. cv. Little Marvel) were sown in vermiculite and germinated in a glasshouse with natural lighting and maintained at 25±2°C. The seedlings were watered with 1/4-strength Hoagland solution for the first 2 weeks and with full-strength solution thereafter. When they were 6 weeks old they were transferred to a growth chamber with a day/night temperature of 25/15°C. The photoperiod was 16 hr (0600-2200) and the light intensity was approximately 400 μE m⁻² s⁻¹. After a 48-hr pre-conditioning period in the growth chamber, the temperature was changed to constant 10, 25, or 40°C.

To prevent water stress as a result of heat-induced dehydration, pots of all treatments were transferred to trays containing full-strength nutrient solution. Holes in the bottom of the pots allowed adequate uptake of solution. The light period was maintained at the same duration and intensity. The leaf water potential of plants was measured with a pressure bomb and did not differ with the temperature treatments. After 24-hr exposure to the treatment, single-plant samples from each chamber were harvested in triplicate. Top portions were harvested, frozen on dry ice and kept in a freezer at about –20°C until analyzed.

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