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HORMONAL REGULATION OF WHEAT GROWTH DURING HYDROPONIC CULTURE

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ABSTRACT

Hormonal control of root growth has been explored as one means to alleviate the crowding of plant root systems experienced in prototype hydroponic biomass production chambers being developed by the CELSS Breadboard Project. Four plant hormones, or their chemical analogs, which have been reported to selectively inhibit root growth, were tested by adding them to the nutrient solutions on day 10 of a 25 day growth test using spring wheat in hydroponic cultures. Growth and morphological changes in both shoot and root systems were evaluated. In no case was it possible to inhibit root growth without a comparable inhibition of shoot growth. It was concluded that this approach is unlikely to prove useful for wheat.

HORMONAL REGULATION OF WHEAT GROWTH DURING HYDROPONIC CULTURE

1. INTRODUCTION

1.1 PURPOSE OF THE PROJECT

Prolonged inhabitation of space will require man to use photosynthetic organisms to supplement food and oxygen needs (1). Initial studies underway in NASA's Controlled Ecological Life Support System (CELSS), are exploring the use of familiar crops plants which will be grown hydroponically (2). Crop plants normally develop extensive root systems which mine the soil for mineral nutrients and water. Although nutrient and water supplies are no longer growth limiting factors in hydroponic cultures, plants continue to produce large root systems. Producing and maintaining such large root systems may use as much as a third of the sugar and oxygen produced by the photosynthetic shoot system. The crowded mats of fiberous roots produced in hydroponic culture may create environmental conditions unhealthy for the roots themselves, and favorable for the growth of undersirable microorganisms. In addition, fiberous roots are generally unacceptable as food without extensive food processing. For all of these reasons we are exploring ways to reduce the size of the root system in relation to the shoot system without reducing the rate and extent of shoot development.

1.2 BACKGROUND INFORMATION

Relatively little is known about the way shoot and root growth are coordinated to produce a structurally and functionally balanced plant. It is believed that chemical communication between the shoot and root system plays a controlling role (3). Plant hormones and related substances are known to be powerful growth regulators and to be transported between shoots and roots. For example, cytokinin hormones are required for shoot growth but are produced mainly in roots. Cytokinins are thought to be transported from root to shoot passively in the transpiration stream. Cytokinins are also powerful inhibitors of root growth, at least when exogenous supplied. Thiamine is essential for root growth yet is produced exclusively in photosynthate shoot tissue. It moves to the root in the phloem photosynthate stream. Auxins strongly inhibit root growth at concentrations which are stimulating to shoot growth. Gibberellins selectively stimulate stem growth. Abscisic acid and ethylene are powerful growth regulators. Undoubtedly the exchange of such growth regulators as these help coordinate the orderly development of shoot and root systems. Many other inorganic and organic molecules and ions are found in transport fluids of the plant. Apart from the obvious dependence of roots on photosynthate and shoots on minerals and water from the root

system little is known about the significance of the other substances in maintaining shoot-root balance.

1.3 EXPERIMENTAL APPROACH

In the absence of a full understanding of the interrelationships of root and shoot, we have taken an empirical approach to determine whether shoot and root growth can be manipulated independently using known growth regulating hormones. We were encouraged to believe that some manipulation of shoot-root ratio is possible by a number of reports in the literature which show that the ratio may vary in response to changes in nutrition (4,5,6), water status (7), and genetic factors (5,6). The method of application of test chemicals was largely determined by our desire to selectively manipulate the growth of the root system, and the fact that the strongly hydrophobic cuticular layer of the shoot greatly impedes passage of water soluble molecules like plant hormones. Presentation of these chemicals directly to the roots circumvents shoot permeability problems and places the chemical at the desired site of action without need for long distance transport. For these reasons, as well as our desire to manipulate hydroponically grown plants, the hormonal substances were dissolved in the hydroponic nutrient solution. We have studied the effects of either the natural hormone or a synthetic analog from each of four known classes plant hormones; auxins, cytokinins, gibberellins, and abscisic acid.

2. MATERIALS AND METHODS

2.1 SEED STERILIZATION AND GERMINATION

Spring wheat (Triticum vulgare cv. Yecora Rojo), obtained from the California Crop Improvement Assoc. Davis, CA, was used as the test plant. Seeds selected for uniformity of size and free from visible damage were surface-sterilized by immersion in 50 ml of a 1% solution of sodium hypochlorite solution containing a drop of neutral detergent (Tween 80 Sigma Chem., Co., P-1754). After 15 minutes, three rinses with sterile water lasting a total of 5 minutes removed most of the residual sterilizing solution and seeds were allowed to germinate for 24 hrs. in darkness at 27C on wet sterile filter paper. Seeds showing seedling emergence at 24 hrs. were aseptically transferred to the culture apparatus.

2.2 DESIGN OF THE CULTURE APPARATUS

Half-liter cylindrical jars (Fisher Sci. Co. 03-320-10E) were modified as shown in Figure 1 to provide hydroponic culture vessels which would hold germinating seedlings in a moist

sterile environment suspended over 400 ml of a sterile nutrient solution. After 3-4 days in darkness seedling roots grew into the nutrient solution and the seedling holder cap (not shown in Figure 1) was aseptically replaced with a soft plastic foam plug. Cultures were then placed in a controlled environment growth chamber.

2.3 ENVIRONMENTAL PARAMETERS

A modified cabinet-style plant growth chamber (EGC Chagrin Falls, OH) provided regulation of light and temperature. A photosynthetic photon fluence rate of 200 u mol m-2sec-1 was provided by overhead fluorescent lamps (110 watt Vita-lite 1500). A 16 hour photoperiod alternated with 8 hours of darkness. Temperature was held at 24 1C during the photoperiod and 20 1C during darkness.

Carbon dioxide and water vapor levels were continuously monitored but not regulated. The mean CO2 concentration was 390 30 ppm during the photoperiod. The relative humidity during the photoperiod was 75 10%. Filtered ambient air was supplied to the root system through a vertical tube (see Fig. 1) and the rising air bubbles served to stir the nutrient solution as well as aerate it.

2.4 THE NUTRIENT SOLUTION

Germinated seedlings were maintained for the first 10 days in 400 ml. of half-strength Hoagland's solution, a standard formulation of mineral nutrients known to be essential for the growth of plants. On day 10 this nutrient solution was replaced with 400 ml. of full strength Hoagland's solution supplemented with 2.5 mM MES (2 [N-Morpholino] ethane sulfonic acid) pH buffer and adjusted to pH 5.8. This buffer was previously tested for hydroponic culture of wheat by Bugbee and Salisbury (8). Nutrient media were filter-sterlized using Gelman mini-capsule filters of 0.45 pm porosity.

2.5 MEASUREMENT OF EXPERIMENTAL DATA

Shoot height, leaf number, leaf length, and tiller number and length were measured with a millimeter scale at 48 hour intervals from day 11 to day 24. Observations of shoot and root morphology were made at each measurement period. On day 25 the plant was removed from culture, severed at the shoot-root juncture and the fresh weights of the parts measured with milligram accuracy. After drying the parts for 18 hours at 85C, dry weights were measured with the same accuracy.

On day 20 2 ml samples of the nutrient solution were taken aseptically from each culture for measurement of microbial

contamination and pH. Microbial contamination was detected and quantified by spreading 0.1 ml of each sample on R2A agar (Difco Laboratories) and counting the number and kind of colonies visible after 48 hours incubation at 27C, Conductivity of the nutrient solutions was measured using a Markson 103 meter.

2.6 TEST CHEMICALS*

The synthetic auxin analog alpha napthaleneacetic acid was used because it is more chemically stable than the natural auxin hormone indoleacetic acid. The synthetic cytokinin analog benzyladenine was used for the same reason. The compound 2-chloroethylphosphonic acid is widely used to produce ethylene in plant tissues. Hormones and related chemicals were purchased from Sigma Chem. Co.. Catalog identification numbers are given in lieu of complete description: alpha napthalene acetic acid #N-0375, 6-benzyladenine #B-6750, gibberellic acid A3 #G-3250, cis-trans abscisic acid #A-1012, 2-chloroethylphosphonic acid #C-0143.

3. RESULTS

3.1 ROOT AND SHOOT GROWTH

All components tested inhibited root and shoot growth to some extent. Accumulated root and shoot biomass and biomass ratios are summarized in Table 1. Selected growth data expressed as shoot height, is presented in Figure 2. Leaf number, leaf length and tiller numbers and length were also recorded at 48 hour intervals. These latter data will not be presented here, however, they are available upon request.

3.2 CHANGES IN ROOT SYSTEM MORPHOLOGY

Extensive modification of root system morphology was observed when NAA, BA, or ABA were added to the hydroponic nutrient. A normal hydroponically-grown wheat root system develops 6 - 10 relatively coarse roots of seminal or adventitious origin which reach lengths of up to 14 inches in 24 days. These major roots give rise, along their entire length, to large numbers of slender lateral roots 2 - 4 inches long on 25 day old plants. Numerous short tertiary roots may also be present. All root surfaces,
except the terminal 1 to 3 cm, are covered with a dense layer of translucent hairs which reach a length of 2 mm in the larger

*Abbrevations used: NAA=Alpha napthaleneacetic acid; BA=6-benzyladenine; GA=gibberellic acid A3; ABA=abscisic acid; CEPA=2-chloroethylphosphonic acid.

roots. Root extention occurs entirely by division and

elongation of cells in this tip region. During normal growth root hair development begins 1 - 3 cm. behind the root tip leaving the elongation region smooth-surfaced.

Benzyladenine, even at 1 x 10^{-9} M (data not shown) caused complete cessation of root extension within 24 hours. Root hair development was accelerated and hairs 2-3 times normal length appeared throughout the normally smooth elongation zone to the edge of the root tip. Inhibition of root extension lasted throughout the 25 day test period, however, slow partial recovery was evident in the last several days and at the lower concentrations some new branch roots were formed. Most inhibited root apices, including the zone of abnormal root hairs, formed unusual crooks and loops which remained throughout the test.

Napthaleneacetic acid also caused abrupt cessation of root extension accompanied by some exaggerated root hair development and tip distortion. However, the major morphological effect was the stimulation of large numbers of latent lateral root primordia throughout the root system. At 1 x 10-6M these primordia broadened abnormally and were inhibited at approximately 1 mm length. At 1 x 10-7M the new primordia developed with normal branch root morphologzy except that elongation did not exceed 1 cm and all lateral surfaces except the tips become covered with abnormally long root hairs. At 1 x 10-7M the inhibitory effect was weakened enough to allow a small amount of further extension of all root tips during the last several days of the test.

Some root hair overgrowth and inhibition of root extension was also observed with abcisic acid. Gibberellic acid caused no morphological abnormalities.

3.3 CHANGES IN THE NUTRIENT SOLUTION

Hydroponic culture using Hoagland-type nutrient solutions increase in pH as growth proceeds. During the 25 day growing period of these experiments pH shifted from the initial set value of 5.8 to terminal values ranging from 6.1 to 6.9 with treatments sustaining the strongest growth reaching the latter value. Terminal values were well within the normal range for healthy plants.

Electrical conductivity of nutrient solution decreases as growth proceeds and nutrients are taken into the plant. Initial conductivity of full strength Hoagland's solution was 2.80 u Siemens cm-1. Terminal values ranged from 2.3 to 2.7 and confirmed that nutrient supplies were not significantly depleted.

In spite of precautions, a low level of bacterial contamination was found in all nutrient solutions by the end of the growth

period. Bacterial counts range from 1-8 x 10^3 per ml and judging from colony characteristics were usually of the same species.

4. DISCUSSION

4.1 ROOT GROWTH

Benzyladenine, napthaleneacetic acid, and abcisic acid all proved to be strong inhibitors of root growth in these tests. These three substances represent fundamentally very different hormones, however, their actions here show surprising similarities.

Although the details differ, all inhibited the linear growth of the root apices while at the same time greatly stimulating the growth of root hairs. The common responses suggest a common mechanism of action. It is known that cytokinins and auxins, among their many actions, promote the formation of ethylene by stimulating the enzyme ACC synthase, a rate limiting step in ethylene biosynthesis in plant cells (9). Two well documented actions of ethylene are the inhibition of stem and root cell elongation and the enhancement of root hair formation from the epidermis (10). It is reasonable to assume that benzyladenine and napthaleneacetic acid have acted in this way. Choroethylphosphonic acid, a compound which is converted into ethylene by plant cells, is currently being tested in this project to aid in confirming this idea. Abscisic acid-treated plants showed some similarities with BA and NAA treated plants, however, interpretation is complicated by its inherent growth inhibiting action and may or not involve ethylene. These data do not support a recent published report of stimulation of root growth by ABA (7). The stimulation of lateral root primordia by NAA is a characteristic action of auxins and results from stimulation of DNA replication and cell division in competent cells. At 5 x 10-6M, the stimulation was so strong that small disorganized cell masses was formed rather than root tips. At 5 x 10-7M root tips emerged from each primordium but were inhibited from elongating to form normal lateral roots. Gibberellic acid caused some reduction in root growth, however, the appearance of the root system differed little from the control. The action of gibberellins in roots is poorly understood, however, it seems fundamentally different from that of the other three hormones.

4.2 SHOOT GROWTH

Although all four compounds reduced shoot growth, both mass and dimentional growth, there were no overt qualitative differences among the treatments. Pigmentation and morphology were normal. Differences in the rate of leaf and tiller formation appear to be related to growth rate rather than organ-specific effects of any hormone treatment. GA3 applied to roots at 3 x 10-6M has been reported to stimulate shoot growth in Pelargonium(11), an effect not observed in this study with wheat.

4.3 SHOOT-ROOT INTERACTION

It is clear from these experiments that there is a strong interdependence between shoot and root growth which is not significantly disrupted by hormonal treatments which reduce root growth. In all treatments in which root growth has been inhibited, shoot growth has also been roughly proportionately reduced. This result is particularly surprising in the case of cytokinin treatment (benzyladenine). Cytokinin supply is thought to be a major limiting factor for shoot growth (3). Rootless shoots can be grown in vitro, but only if cytokinins like benzyladenine are supplied in the culture medium. Our present crude understanding of shoot-root interaction would predict that root growth inhibition by any means might also reduce root biosynthesis of cytokinins and in this way reduce the supply to the shoot. It was anticipated, however, that exogenously supplied BA would enter the root and be transported to the shoots compensating for a reduction in endogenous hormone supply. The fact that this did not appear to happen may mean that the synthetic cytokinin analog is not transported in the same way as the endogenous hormone. Alternatively, other as yet unidentified root-synthesized substances required by shoots, may be involved.

5. SUMMARY AND CONCLUSIONS

Experiments were conducted to test the idea that shoot-root ratio could be manipulated by supplying growth regulating hormones, or their analogs, directly to roots during hydroponic culture. A wide range of concentrations of benzyladenine, napthaleneacetic acid, gibberellic acid A3, and abcisic acid were tested on young wheat plants.

Results to date indicate that this approach is not effective. In no case was it possible to inhibit root growth without also inhibiting shoot growth. It is clear from these experiments that the coordination of shoot and root growth may be more complex than anticipated. If we hope to manipulate this relationship, studies of a more fundemental nature may be required. For example, present technology permits the measurment of the very low endogenous concentrations of all known hormones. Tracking concentrations of these hormones in the shoots while root systems are progressively inhibited by non-toxic, non-destructive treatments (eg. lowered root temperature) may enable us to identify a growth limiting hormone. It is also possible that placing exogenously supplied hormones directly on shoot tissue will be more effective than root treatment. Recently Carmi (12) has increased shoot-root ratio in bean plants by applying BA directly to primary leaves. Shoot growth remained unchanged while root growth was suppressed.

It may also be possible to use nutritional or water stress to achieve the desired objective, however, undesirable side effects may limit the usefulness of this approach. In the long run genetic rather than physiological manipulation may prove the more useful approach. Genetic selection has long been an effective way to match plant characteristics to specific environmental conditions. It should not be surprising that food plants which have been selected for centuries, for high performance in terrestrial environments, are difficult to manage in the drastically different conditions of prototype growth chambers designed for use in space.

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TABLE 1. Wheat shoot and root weights, and shoot/root weight ratios after 25 days of hydroponic culture, the last 13 days of which were in the presence of different plant growth regulators. See text for key to abbreviations. Weight data are mean ±s.d., N=2. L.S.D. for shoot/root ratios is ± 15%.

TREATMENT	FRESH WEIGHTS (g)		DRY WEIGHTS (g)		SHOOT/ROOT RATIOS	
	SHOOT	ROOT	SHOOT	ROOT	FRESH	DRY
control	4.05±0.6	3.23±0.4	0.52±.05	0.19±.02	1.3	2.5
BA $4x10^{-6}$M	1.38±0.2	0.75±0.1	0.24±.04	0.12±.02	1.8	2.0
BA $4x10^{-7}$M	2.69±0.2	1.99±0.2	0.39±.01	0.20±.01	1.4	2.0
BA $4x10^{-8}$M	2.41±.04	1.95±.01	0.37±.01	0.17±.01	1.2	2.1
NAA $5x10^{-6}$M	0.94±0.1	1.16±0.1	0.16±.01	0.12±.01	0.8	1.3
NAA $5x10^{-7}$M	1.04±0.2	1.21±0.1	0.17±.03	0.11±.01	0.9	1.5
GA $3x10^{-6}$M	2.67±0.4	2.76±0.3	0.33±.03	0.15±.02	1.0	2.2
GA $3x10^{-7}$M	2.96±0.1	2.17±0.1	0.37±.01	0.13±.01	1.4	2.9
ABA $1x10^{-5}$M	0.68±0.1	0.65±0.1	0.11±.03	0.05±.03	1.1	2.1

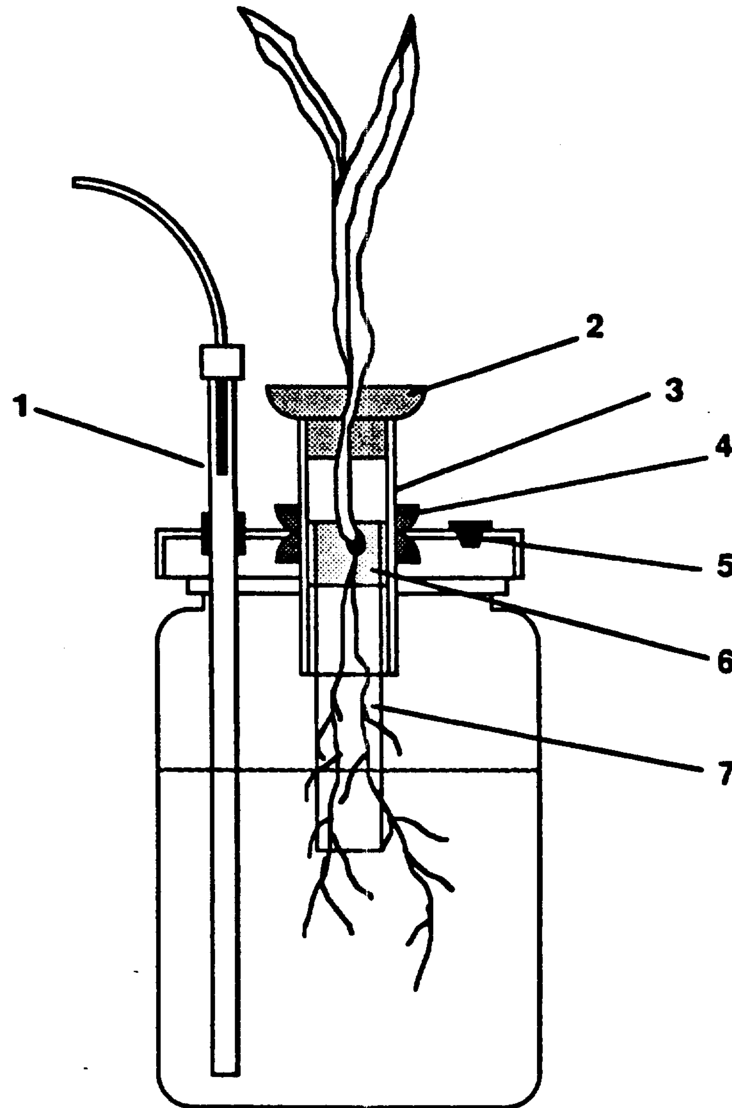

Figure 1. Vertical section of the glass hydroponic culture vessels designed for these experiments. Vessel volume is 500 ml. Vessel is cylindrical with a diameter of three inches. Key to diagram: 1 - aeration tube, 2 - foam plug, 3 - glass cylinder, 4 - foam gasket, 5 - injection port, 6 - foam seed holder, 7 - filter paper wick.

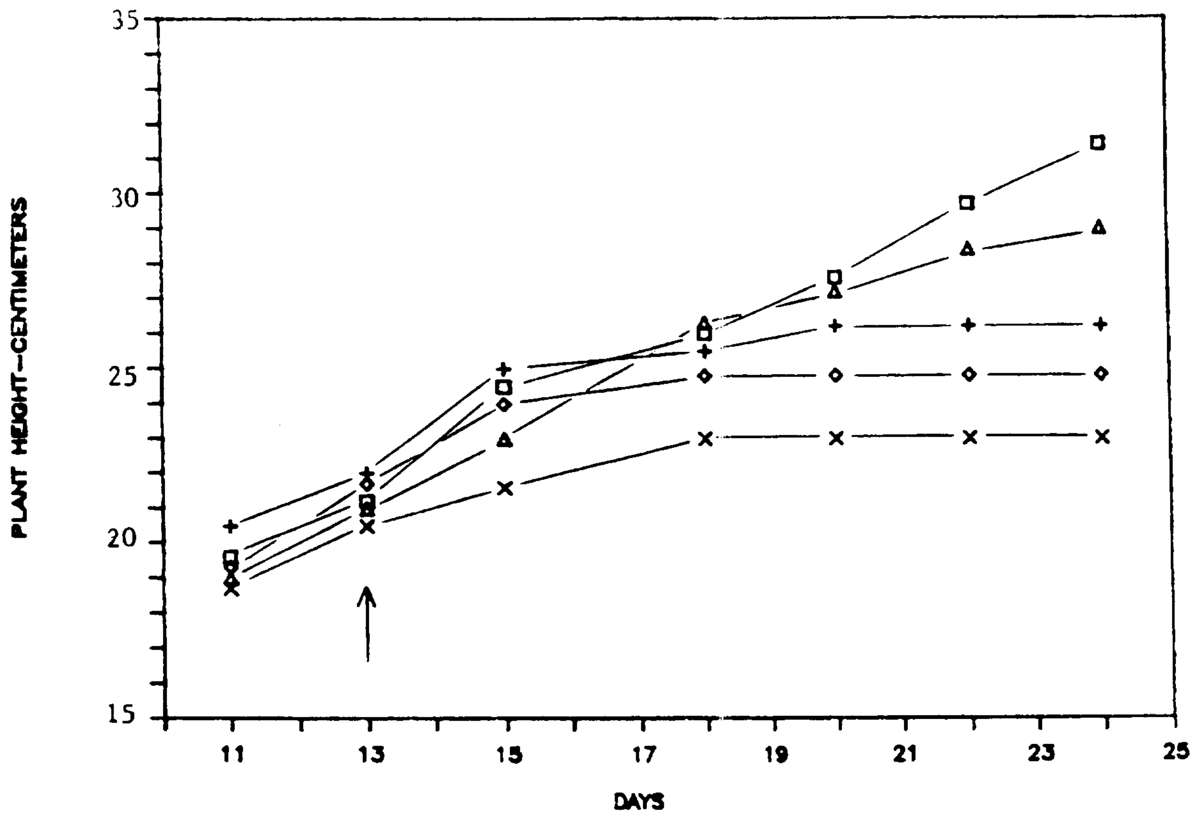

Figure 2. Representative data for growth in height of wheat plants treated with different plant growth substances. Arrow indicates the time that treatments begin. Key to symbols: □ control; △ gibberellic acid A3, 3 x 10-6M; + benzyladenine 4 x 10-6M; ◇ napthaleneacetic acid 5 x 10-6M; x abscisic acid 1 x 10-5M