DISTRIBUTION OF BORON IN CELLS OF DICOTYLEDONOUS PLANTS IN RELATION TO GROWTH[1,2]

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Relatively little is known about the locus of boron in the plant cell or its availability for movement from one site to another. It appears that little or no reutilization of boron occurs in dicotyledonous plants, and an external available supply is needed throughout the period of growth. As the borate ion complexes readily with various polyhydroxy compounds (13), localization of boron in certain cellular fractions might not necessarily imply an important physiological role at these sites; nevertheless, information on its distribution or localization within the plant cell could contribute to a better understanding of its function.

Boron determinations on various intracellular fractions separated by differential centrifugation indicated certain consistent trends regarding the distribution of boron within the cell, particularly in relation to growth, boron utilization and boron availability. These results have been briefly summarized (12) and will be reported fully here.

MATERIALS AND METHODS

Sunflower (*Helianthus annuus* L., var. Mammoth Russian) and mung bean (*Phaseolus aureus* Roxb.) were grown in nutrient solutions in a controlled-environment room under conditions previously described (10). The seeds were sown in quartz sand, watered with tap water, and one week later the seedlings were transferred to nutrient solutions contained in wide-mouth, low-boron soft glass quart fruit jars wrapped in black cloth. The sunflower plants were grown in solutions of the macronutrient composition previously described (10); the solutions for the mung beans consisted of a one-eighth concentration of Hoagland and Arnon's no. 1 solution (3). Iron, manganese, zinc and copper were added to both solutions at concentrations of 5.0, 0.5, 0.05 and 0.02 ppm respectively.

As plants absorb boron somewhat in proportion to the amount available, boron may accumulate at certain sites in amounts greater than required, and it may also be fixed at sites where it is not normally required. For these reasons boron was supplied in amounts that did not exceed normal growth needs. Preliminary studies were conducted to determine the minimal amounts of boron required to maintain normal growth (free of boron deficiency symptoms) for a period of 18 days. These studies indicated that a total of 50 μg boron per plant for sunflower and 5 μg boron per plant for mung bean (supplied as H_3BO_3) was just sufficient for this period of growth under the environmental conditions indicated. At the 18th to 19th day under this regime the sunflower plants started to show symptoms of boron deficiency.

Plants were harvested at several periods for various determinations. Twenty-five g fresh tissue samples were used; this included the terminal bud regions and the blades of the two youngest, fully expanded leaves of about 11 plants for sunflower and the terminal bud regions and the leaflets from the youngest, fully expanded leaf of about 45 plants for mung bean. The samples were prepared and separated into various intracellular fractions by the procedure used by Gordon (2). The tissue was ground in a chilled mortar with the aid of acid-washed sand in 50 ml phosphate buffer, 0.2 M, of pH 7.0, made 0.3 M with sucrose. The homogenate was strained through 200-mesh nylon cloth; the material that passed through is designated homogenate filtrate and that remaining in the cloth as homogenate residue (table I). The various cellular components were separated from the homogenate filtrate by differential centrifugation (table II).[4] Centrifugations up to $17{,}000 \times G$ were performed in a refrigerated model Sb-1 International centrifuge; the $105{,}000 \times G$ centrifugation was carried out in a Spinco Model L preparative centrifuge.

Aliquot samples of the supernatant fraction remaining after centrifugation at $105{,}000 \times G$ for 30 minutes were dialyzed (in cellulose tubing, Visking Company) against 1×10^3 volumes of distilled water at 3° C for 24 hours to remove the dialyzable boron.

Boron analyses were performed by the colorimetric method of Dible et al (1); readings were taken on a Beckman DU spectrophotometer at 540 mμ. Dry weights of the samples were obtained after drying at 100° C for 24 hours and corrections were made for sand and added sucrose.

RESULTS AND DISCUSSION

DISTRIBUTION OF BORON IN INTRACELLULAR FRACTIONS: Two exploratory trials were conducted which involved boron determinations by spectrographic analysis of various cellular fractions separated by differential centrifugation. These results were con-

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[4] Identification of these particulate fractions with the corresponding centrifugations had been made previously by Dr. S. A. Gordon employing light and electron microscopy supplemented with Feulgen and Janus Green B reactions. The mitochondrial fractions were able to carry on oxidative phosphorylation with a P/O ratio of ca. 0.9.

TABLE I

BORON CONTENT OF HOMOGENATE FRACTIONS OF LEAVES AND APICAL BUD REGIONS OF MUNG BEAN AND SUNFLOWER PLANTS *

FRACTION	MUNG BEAN				SUNFLOWER			
	DRY WT OF FRACTION (% OF BOTH FRACTIONS)	BORON CONC OF FRACTION (μG/G)	TOTAL BORON OF FRACTION (μG)	TOTAL BORON IN FRACTION (% OF TOTAL IN BOTH FRACTIONS)	DRY WT OF FRACTION (% OF BOTH FRACTIONS)	BORON CONC OF FRACTION (μG/G)	TOTAL BORON OF FRACTION (μG)	TOTAL BORON IN FRACTION (% OF TOTAL IN BOTH FRACTIONS)
Homogenate residue	61.3	36.1	87.5	49.4	63.3	26.1	67.5	50.1
Homogenate filtrate	38.7	48.1	89.7	50.6	36.7	38.0	67.3	49.9

* 25 g fresh plant sample.

sistent with those of a third and major experiment which will be presented. The boron analyses of all but the two exploratory experiments were carried out by the colorimetric (curcumin) method.

The tissues for this set of analyses were obtained from a harvest made 18 days after the plants were transferred to nutrient solutions containing a total of 50 μg B (for sunflowers) and 5 μg B (for mung beans) per plant. In both the mung bean and sunflower samples the homogenate residues constituted slightly more than 61 % of the total dry matter and contained about 50 % of the total boron (table I). The filtrates had about the same amount of total boron but a smaller dry weight, and therefore contained a higher boron concentration.

Because of overlapping of particle sizes, it is not possible to attain an absolute separation of the various cellular components by differential centrifugation. Nevertheless, boron analyses on the centrifuge fractions (characterized by the indicated cellular components) (table II) showed a variation in boron content. The concentrations and total amounts of boron in the fractions containing plastids and nuclei and in the non-particulate cytoplasm (dialyzed supernatant) were considerably higher than those in the mitochondria or "microsomes." The relatively high concentrations of boron in the chloroplasts is in agreement with the observations of Otting (6) but opposed to those of Smith (9).

Smith (9) separated chloroplasts from squash leaf tissue and found them to contain relatively small amounts of boron; he concluded that this element could have no function in this part of the cell, whereas Otting (6), on the other hand, found considerably higher amounts of boron in chloroplasts. The possible significance of the marked difference between the boron content of the sunflower and mung bean "microsomes" fraction will be referred to later.

The relatively low level of boron in the mitochondria, a fraction which constituted the highest percentage of dry weight of the particulates, is of particular interest in the light of the importance of these structures as sites of enzymatic activity.

These determinations indicate that there is a meas-

TABLE II

BORON CONTENT OF INTRACELLULAR FRACTIONS OF HOMOGENATE FILTRATE OF LEAVES AND APICAL BUD REGIONS OF MUNG BEAN AND SUNFLOWER PLANTS *

INTRACELLULAR FRACTION	MUNG BEAN				SUNFLOWER			
	DRY WT OF FRACTION (% OF ALL FRACTIONS)	BORON CONC OF FRACTION (μG/G)	TOTAL BORON OF FRACTION (μG)	TOTAL BORON IN FRACTION (% OF TOTAL IN ALL FRACTIONS)	DRY WT OF FRACTION (% OF ALL FRACTIONS)	BORON CONC OF FRACTION (μG/G)	TOTAL BORON OF FRACTION (μG)	TOTAL BORON IN FRACTION (% OF TOTAL IN ALL FRACTIONS)
Cell debris 100 × G; 10 min	41.1	22.9	16.0	30.0	16.0	32.4	8.4	21.2
Nuclei and plastids 1,000 × G; 10 min	8.8	63.7	9.6	17.9	15.3	32.4	8.0	20.2
Plastids and nuclei 4,500 × G; 10 min	13.0	40.3	8.9	16.7	18.8	28.7	8.7	22.0
Mitochondria and plastids 17,000 × G; 30 min	13.9	16.6	3.9	7.4	21.5	10.6	3.7	9.3
"Microsomes" 105,000 × G; 30 min	8.0	24.9	4.0	7.5	11.7	0.6	0.1	0.4
Dialyzed supernatant	15.2	41.3	11.0	20.5	16.7	39.0	10.6	26.9

* 25 g fresh plant sample.

urable difference in the distribution of boron within the cell, and this differential distribution could be related to physiological boron-dependent activities. There are, however, several possible alternative interpretations that would prevent drawing unequivocal conclusions in this regard, namely 1) the localization of boron may reflect the distribution and differential concentration of polyhydroxy compounds that complex with boron, 2) since the fractionations were carried out in an aqueous medium, some dissociation of the boron complexes may have occurred, and 3) nonspecific adsorption of boron by certain particulates may have taken place during the grinding and isolation procedures and therefore might not reflect boron distribution in an intact cell.

Boron of the Supernatant Fraction: The previous experiment indicates that there is an observable difference in the boron content of the various intracellular fractions. At the present time, however, no definite conclusions can be drawn as to the significance of these findings. A seemingly more significant relationship, however, was suggested when it was noted that certain changes in the boron level of the supernatant fraction took place and appeared to be related to boron availability and boron deficiency. An experiment was then carried out to examine these changes in more detail.

Sunflower plants, of sufficient numbers to provide daily harvest requirements over an extended period, were grown in nutrient solutions containing a total of 50 μg B per plant. Samples were prepared in the usual manner except that only the supernatant fraction remaining after centrifugation at 105,000 × G for 30 minutes was used. Boron determinations were made on aliquots of the total supernatant and on aliquots of dialyzed supernatant, thus providing boron analyses on 1) total supernatant, 2) nondialyzable supernatant (the supernatant aliquot after dialysis) and 3) dialyzable supernatant (by difference between 1 and 2).

Plants were harvested daily from the early part of the experiment until pronounced boron deficiency symptoms had developed. At the early part of the experiment when the plants were making normal growth, the nondialyzable boron represented about 25 % and the dialyzable boron about 75 % of the total supernatant boron (fig 1). As boron was exhausted from the nutrient solution, the dialyzable boron fraction of the supernatant dropped and finally reached zero when the plants exhibited pronounced boron deficiency symptoms at the terminal buds. Smith (9) also noted that the boron in the "vacuolar sap" declined to a very low level when the plants were boron-deficient. The nondialyzable boron fraction of the supernatant, however, remained constant throughout this time. These findings illustrate that the small amount of boron in the plant which is available for required plant functions is the unbound portion in the supernatant. When this is depleted, the plant exhibits the usual deficiency symptoms.

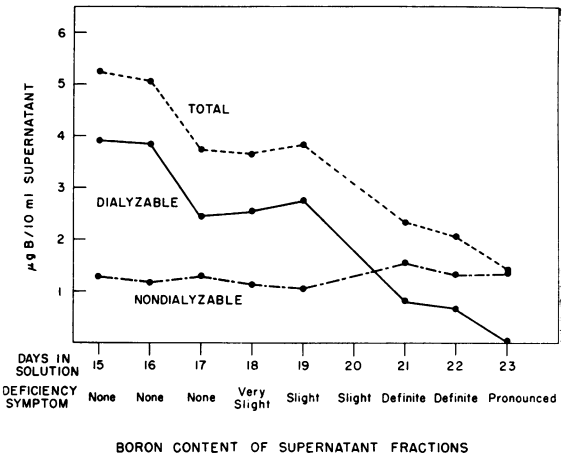

Fig. 1. Boron content of supernatant fractions (following centrifugation at 105,000 × G for 30 minutes) of sunflower tissue obtained from plants grown with a total of 50 μg B per plant for various periods.

The bound portion is apparently not available for movement to other sites. These observations also further substantiate various previous observations (e.g., 11) that boron is not reutilizable.

It appears that the dialyzable portion of the supernatant fraction represents free boron not yet utilized. Although it is of great importance to the economy of the plant, it may not serve any special physiological function in itself but perhaps represents an available pool for use in specific functions.

The physiological importance of the soluble boron fraction (cell sap obtained by mechanical pressure) in plants has been pointed out (4, 5, 7, 8) but this fraction has generally not been further separated. Shive (8) has shown that monocotyledonous plants contain greater amounts of soluble boron than do dicotyledonous species which is perhaps the reason why the former usually take longer to show pronounced deficiency symptoms.

Attention has been called to the marked difference between the boron content of the sunflower and mung bean "microsomes" fraction (table II). Sunflower plants grow considerably more vigorously than do mung bean plants and by the 18th day (time of harvest) and shortly thereafter, incipient boron deficiency symptoms generally appear in plants supplied with a total of 50 μg B per plant. Although the mung beans received one-tenth this supply, their rate of boron utilization is considerably slower and it is quite possible that they had not reached the same stage of incipient boron deficiency. The boron in the "microsomes" fraction may possibly be lightly bound and subject to release when the dialyzable supernatant boron level declines. If this is true it would be of considerable interest as other fractions of bound boron are apparently not available for further physiological utilization.

SUMMARY

Boron determinations were made on intracellular fractions separated by differential centrifugation. Mitochondria and "microsomes" were found to be lower in boron than nuclei, plastids and dialyzed supernatant fractions.

The boron content of the nondialyzable supernatant fraction does not change in response to decreasing amounts of available boron accompanied by the appearance of boron deficiency symptoms. The boron content of the dialyzable portion of the supernatant fraction, however, declines when supplies of available boron decline and it finally reaches zero in this fraction when plants exhibit pronounced boron deficiency symptoms.

We wish to thank Dr. S. A. Gordon for his valuable help and advice on the separations of the intracellular fractions by differential centrifugation and Messrs. J. K. Brody and J. A. Goleb for carrying out the spectrographic analyses.

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ON THE ACTION OF PLANT GROWTH REGULATORS. II. ADSORPTION OF MCPA TO PLANT COMPONENTS[1]

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Plant growth regulators may be defined to suit the purposes of this paper as substances which at low concentrations give rise to increased cell elongation but at higher concentrations to phytotoxicity. The two effects may be quite distinct. The following paper offers a contribution to the knowledge of the mechanism of selective phytotoxicity in established plants.

Plants vary considerably in their resistance to growth regulators. Grasses and cereals generally, are resistant to low doses of 2-methyl-4-chlorophenoxyacetic acid (MCPA). Rape and mustard are highly susceptible whereas other species such as groundsel, chickweed and clover are intermediate. Resistant plants however respond to higher doses of MCPA. It may be assumed therefore, that a physiological effect can be produced in all plants provided the plant growth regulator reaches the appropriate site in sufficient quantity.

Resistance may result from one or more of the following causes: (a) low penetration of the solution into the plant; (b) adsorption losses during translocation from the leaf or stem to the site responsible for the physiological effect; (c) rapid destruction of the growth regulator by the plant; (d) an adverse effect on the permeability of cell membranes; and (e) low adsorption to sites responsible for the physiological effect.

In this work, adsorption processes are considered which could occur after the regulator has entered the plant. Thus it was shown (1) that there is a strong

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