Quantification of Abscisic Acid in a Single Maize Root

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ABSTRACT
Quantitative analyses of abscisic acid in the elongating zone of a single maize root (Zea mays L. cv LG 11) were performed by gas chromatography-mass spectrometry using negative chemical ion ionization. Data showed that the endogenous acid, the slower the growth, but a large dispersion of individual values was observed. We assume that abscisic acid is perhaps not correlated only to the growth rate.

ABA has been found in the cap (7) and in the entire maize root (5, 9). Applied ABA moves preferentially in a basipetal direction thus inducing growth inhibition (3); however, a small but significant growth stimulation (1, 4) may occur at low concentration.

The large variability in growth rates observed in a root population (6, 11) has been correlated to both endogenous IAA (6) and ABA (8) content of growth classes. However, the relative low sensitivity of the detection of ABA-methyl derivative with MS in electron impact mode has until now made the analysis of very small samples difficult. At least 20 root segments were used for each determination (8); this induced a randomization of the information given by the individual growth rate and consequently a decrease in the degree of correlation.

The aim of this work was, using NCI,1 to determine the ABA content, expressed as a function of the growth rate, in the elongation zone of a single root of Zea mays. The possible role of endogenous ABA in growing maize root is reexamined.

MATERIALS AND METHODS
Plant Material. Germination of selected caryopses of Zea mays (cv LG 11) was previously described (2) and adapted to the present experiments. Primary roots attached to their caryopses elongated vertically on moist paper towels between two transparent plastic frames. Photographs of the frames were taken at 48, 51, and 54 h after imbibing the seeds (blue flash at minimum energy) and the frames were then immediately stored at −80°C. Root length was measured on photocopied films by using a digitizing pad (Hi Pad, Houston Instruments, Austin, TX) interfaced with a microcomputer (ABC 80, Luxor AB, Motala, Sweden). After the growth measurement, parts of roots corresponding to the elongating zones (2.5–5 mm counted from the tip) were individually harvested, freeze-dried, and stored.

ABA Extraction and Analysis. The general procedure was described elsewhere (8) and adapted to the extraction and analysis of endogenous ABA in a single root segment (about 2 mg fresh weight). One ng of hexadeuterated-(±)ABA was added to the segment as an internal standard at the beginning of the procedure. Each segment was allowed to equilibrate for 5 min in 1 ml of phosphate buffer (1/3 M, pH 8.0, 4°C). The solution was then washed with 1 ml petroleum ether. After acidifying the buffer to pH 3.0 with 2 N HCl, the organic acids were extracted with 1 ml of diethylether. The ether phase was evaporated to dryness (N2 stream, 20°C) and the residue methylated with diazomethane (50 μl methanol, 500 μl ethereal diazomethane). After 10 min the reagents were eliminated by evaporation (N2 stream, 20°C), the resulting methylester dissolved in 10 μl hexane and stored at −30°C.

The analyses were done by a GC-MS model Hewlett-Packard 5985 A equipped with NCI. Splitless injections of 3 μl were made onto a SE 54 WCOT fused silica capillary column (25 m long, 0.3 i.d.). Typical GC conditions were: injector 250°C, GC oven 100°C programmed at 25°C min−1 to 240°C, He approximately 1.5 ml min−1. The MS conditions were: ionization potential 150 eV, ion source 200°C. The value of the M+ was monitored (m/z = 278 and 284 for ABA-methyl and hexadeuterated ABA-methyl, respectively) and the level of endogenous ABA was calculated from a calibration curve with a regression coefficient higher than 0.995.

[Graph and data table]

Fig. 1. Reconstructed SIM trace at m/z 278.1 and 284.1 using ammonia NCI. Sample from a single Z. mays root extract was injected (fresh weight about 2 mg) and molecular ions of ABA-methyl and hexadeuterated (±)ABA-methyl were monitored. GC-MS system was set at maximum detector gain (3000 eV).

1 Abbreviation: NCI, negative chemical ion ionization.
RESULTS AND DISCUSSION

The good selectivity and sensitivity of the method is illustrated for one individual analysis in Figure 1. The mean percentage recovery of ABA was 40%. The signal of methylated-ABA is well above the background, indicating that one could even determine the ABA content in a fraction of the elongating zone.

Values of endogenous ABA, as a function of the growth rate, are given for individual roots in Figure 2. A correlation curve was calculated from the inverse values of ABA level expressed as a function of the growth rate. These data clearly indicate, at least for the growing part of maize root, that a higher ABA level is related to a lower growth rate. Results reported in a previous paper (8) where ABA level was measured in groups of 30 roots (growth classes) are thus confirmed.

However, these individual values show a large dispersion which could not be revealed by measurement in groups of roots and which is not inherent to the small amount of material used (Fig. 1). We may assume that the ABA content in the elongating zone is not only correlated to the growth rate, but also to some other parameters which might influence its endogenous level, like the number and the sizes of the cells in the elongating zone, the density of the vacuoles, the presence of some other growth regulators, and so forth.

It would, therefore, be interesting to express the amount of ABA in the elongating zone with respect to several of these physiological and cytological parameters. Surely this technique has the advantage of making such an experiment possible.

Moreover, data presented here lead us to reexamine the role of ABA in the root elongation. If the endogenous level of ABA is expected to modulate the growth rate, then, according to the shape of the curve (Fig. 2), its inhibitory effect will change along the range of growth rates. For slowly growing roots (0.4–0.7 mm·h⁻¹) a small difference in the elongation rate is correlated to a large variation of the ABA level, whereas for the rapidly growing roots (0.7–1.4 mm·h⁻¹) the increase in the growth rate is only followed by a small change in the ABA content. This could indicate that the 'sensitivity' of the roots to the hormone is dependent on their growth rate. Such a concept has been previously discussed (10). On the other hand, when looking at the values distribution (Fig. 2), it could be supposed that ABA has no direct effect on the growth rate. Indeed, the ABA level of slowly growing roots (0.4–0.7 mm·h⁻¹) covers the entire range of values and it is difficult to correlate it to the growth rate, whereas the ABA level of rapidly growing roots (0.7–1.4 mm·h⁻¹) has a nearly constant value independent on the growth rate.

Further experiments have to be done to decide what could be retained out of these two possible explanations. The determination of the extractable part of ABA really involved in growth processes is necessary. The analysis of growth effect of applied ABA on individual root would also bring useful information on the role of this hormone in the elongation process.

In conclusion, we have shown that, using NCI, the quantification of ABA in a single maize root is now possible. Results indicated that the ABA content of maize roots is perhaps not correlated only to the growth rate.

LITERATURE CITED

8. RIVIER L, M SAUGY 1986 Chemical ionisation mass spectrometry of indol-3-acetic acid and cis-abscisic acid: evaluation of negative ion detection and quantification of cis-abscisic acid in growing maize roots. J Plant Gr Regul 5: 1–16

Fig. 2. Changes in the amount of ABA (in picograms) in the elongating zones (2.5–5 mm counted from the tip) of single roots as a function of their growth rate; 71 maize roots were analyzed. Correlation curve was calculated from the inverse ABA values. The regression equation is: 

\[ y = 1/(0.0297x - 0.0057); \] 
correlation coefficient: 0.65.