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Chemical Ionization Mass Spectrometry of Indol-3yl-Acetic Acid and Cis-Abscisic Acid: Evaluation of Negative Ion Detection and Quantification of Cis-Abscisic Acid in Growing Maize Roots

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Abstract. Mass spectra of the derivatives of indol-3yl-acetic acid and cis-abscisic acid were obtained in electron impact and chemical ionization positive ion and negative ion modes. The respective merits of methane, isobutane, and ammonia as reagent gases for structure determination and sensitive detection were compared using the methyl esters. From one to 10 fluorine atoms were attached to IAA to improve the electron-capturing properties of the molecule. The best qualitative information was obtained when using positive ion chemical ionization with methane. However, the most sensitive detection, with at least two ions per molecule, was achieved by electron impact on the IAA-HFB-ME derivative and by negative ion chemical ionization with NH_3 on the ABA-methyl ester derivative.

Quantitative analyses of ABA in different parts of maize (*Zea mays* cv. LG 11) root tips were performed by the latter technique. It was found that the cap and apex contained less ABA than the physiologically older parts of the root such as the elongation zone and the more differentiated tissues. This technique was also used to show a relation between maize root growth and the endogenous ABA level of the elongation zone and root tip: there is more ABA in the slowly growing roots than in the rapidly growing ones.

Abbreviations: ABA, cis-abscisic acid; BSTFA, bis (trimethylsilyl) trifluoroacetamide; CI, chemical ionization; ECD, electron capture detection; EI, electronical impact; FID, flame ionization detection; GCMS, gas chromatography–mass spectrometry; HFBI, heptafluorobutyrylimmidazole; IAA, indol-3yl-acetic acid; NCI, negative-ion chemical ionization; NPD, nitrogen-phosphorus selective detection; PCI, positive-ion chemical ionization; SIM, selected ion monitoring; TFAA, trifluoroacetyl anhydride; TIC, total ion current; TMCS, trimethylchlorosylane.

Selected ion monitoring (SIM) is now recognized as the best technique for measuring trace amounts of plant growth regulators when a gas chromatograph–mass spectrometer (GCMS) and stable isotope internal standards are available (Morgan and Durham 1983). Cis-abscisic acid and the halogenated derivatives of indol-3yl-acetic acid have strong electron affinities (Seeley and Powell 1974, Bittner and Even-Chen 1975, Hofinger 1980), and this property allowed us to test the use of high-pressure chemical ionization (CI) for the efficient production of anions. The development and evaluation of CI mass spectrometry using positive-ion (PI) and negative-ion (NI) modes for IAA and ABA are presented. The influence of the choice of the derivative and the reagent gas on the structural information obtained and on the sensitivity of detection is discussed. Results of the quantification of ABA in a particular biological sample are compared for the various modes of ionization.

It is now clear that ABA is one of the inhibitory regulators involved in the growth of maize roots (Pilet 1977, Audus 1983). ABA is present in the roots of several species (Kundu and Audus 1974, Hartung and Abou Mandour 1980). It was detected in maize root caps (Rivier et al. 1977, Feldman 1981) and in the root tips of several varieties (Suzuki et al. 1979, Pilet and Rivier 1980, Rivier and Pilet 1981). It is claimed that ABA inhibits the elongation of *Zea* roots (Pilet and Chanson 1981, Pilet and Rebeaud 1983), although some reports are contradictory (Mulkey et al. 1983). To obtain a better understanding of the role of endogenous ABA in the physiology of the maize root, two sets of experiments were done. First, the ABA content of different parts of the maize root was measured. Second, growth classes from a population of roots were chosen and their endogenous ABA level was determined. The relation between a physiological event and an endogenous hormone level can only be established if the same material is used for all assays under the same conditions (light, humidity, temperature) as has already been done for IAA (Pilet and Saugy 1985). For this purpose, small amounts of plant tissue and the highly specific and sensitive negative-ion chemical ionization (NCI) method of quantification were used.

Materials and Methods

The GCMS system consisted of a Hewlett-Packard 5985A GCMS equipped with the NCI option. The same system was used and described in a previous paper (Pilet and Saugy 1985). The ionization potential was 70 eV with 1.5 mA emission for PCI and NCI. The reagent gases were introduced through the direct inlet probe aperture, and ion source pressures could be evaluated by the Pirani gauge connected to the same aperture. The source pressure for normal electron impact (EI) experiments was 6×10^{-6} Torr, while for CI it was between 8×10^{-5} and 10^{-4} Torr, depending on the gas used. In the ion source volume, the pressure of methane was set at 0.5 Torr, isobutane at 0.7 Torr, and ammonia at 0.9 Torr in order to obtain the highest signal from the calibrator, benzophenone.

The quadrupole scan time for EI and CI was 1.2 s for an atomic 300 amu range. Quantification was undertaken using the stable isotope dilution method previously described (Rivier and Pilet 1982). All compressed gases were obtained from Carbagaz, Lausanne, at 99.995% purity or higher. IAA and ABA

were from Fluka, Buchs, Switzerland, and trans-ABA was isolated by TLC after photoisomerization of ABA in methanol. 5-Fluoro-IAA was obtained from EGA-Chemie, Steinheim, East Germany.

Formation of the Derivatives

The derivatizations were done with 500 ng of IAA or ABA in microvials under dry conditions. (1) Methyl esterification was carried out with diazomethane in Et_2O (Rivier and Pilet 1974). (2) Trimethylsilylation was performed with 100 µl BSTFA containing 1% TMCS in 500 µl pyridine at 75°C for 45 min. Such conditions give the bis-TMS derivative of IAA. With ABA, it was not possible to obtain a single derivative. (3) Pentafluorobenzyl esterification followed the procedure described by Epstein and Cohen (1981). (4) Trifluoroacetylation was quantitative using TFAA and IAA-methyl ester at 60°C for 3 h (Seeley and Powell 1974). (5) Acylation with HFBI has already been evaluated critically by Allen et al. (1982), and their method was used without modification. (6) Pentafluoropropionylation of the carboxyl and amine groups was performed with a mixture of PFP-anhydride and PF-propanol (4:1) in order to obtain simultaneous esterification and N-acetylation of IAA (Allen and Baker 1980).

The yields of the derivatizations were measured by GC-flame ionization detection by following the disappearance of the starting material in the case of IAA-Me and/or the appearance of the derivative. No further attempt to optimize the reactions was made, so the yields obtained so far may not all be maximum values. After the reagents had been removed by evaporation under vacuum, the derivatives were dissolved in 100 µl acetone or n-hexane and injected without further purification.

Maize Seedlings

The germination of selected caryopses of *Zea mays* cv. LG 11 (Association Suisse des Sélectionneurs, Lausanne) has been described previously (Pilet 1977). Intact seedlings with rectilinear primary roots (16 ± 0.5 mm in length) were used for all the experiments. Preparations and manipulations were carried out in dim green light (530 ± 20 nm).

Plant Materials (Longitudinal Gradient)

In the first set of experiments, apical segments of length 15 ± 0.1 mm were cut into six sections: the cap (0–0.5 mm), the apex (0.5–1.0 mm), an intermediate zone (1.0–2.5 mm), the elongation zone (2.5–5 mm), and two sections (5 mm in length) into the differentiated tissue. This material was immediately freeze-dried for 18 h and stored (−80°C, in the dark) until the extraction.

Plant Materials (Growth Classes)

In these assays, intact seedlings were placed vertically for 8 ± 0.1 h in plastic frames. The frames were kept in closed boxes in humid air (90 ± 5% relative

humidity) at a controlled temperature (21 ± 1°C). Black-and-white photographs (Ilford FP 4, Yashika) were taken initially and 8 h later (Pilet and Saugy 1985). The films were photocopied using a microfilm reproducer (×10). For growth analysis the roots were measured on the photocopies (Pilet et al. 1983) by using a digitizing pad (Hi Pad, Houston Instruments, Austin, TX, USA) interfaced with a microcomputer (ABC 80, Luxor AB, Motala, Sweden). The elongation zone designated as 2.5–5 mm from the tip (Versel and Mayor 1985) and the root tip (0–2.5 mm) were separated. Corresponding segments were stored individually in PVC microbiological test plates at −80°C. The segments were grouped according to selected growth classes and kept in the dark (−80°C) until analyzed for endogenous ABA. The mean growth rate of the "control" (C) roots was obtained by measuring the difference in their length between 36 h and 60 h during the germination conditions.

Extraction of ABA

The procedure of extraction and quantitative determinations were performed basically by using the stable isotope dilution technique previously described (Rivier et al. 1977, Pilet and Rivier 1980). It was possible to simplify the procedure further because of the higher specificity of the NCI detection method compared with EI-GC-MS used in the preceding works. Twenty nanograms of hexadeuterated-(±)ABA (Rivier et al. 1977) was added as an internal standard to the collected segments (30 per segment) at the beginning of the procedure. Three milliliters sodium acetate/acetic acid buffer (1/15 M, pH = 4.0) was added to the plant material at 4°C, and the samples were allowed to equilibrate for 5 min. They were then purified with 3 ml n-hexane, and the organic acids were extracted with 5 ml $CHCl_3$. The chloroform layer was evaporated to dryness in a vortex evaporator. The residue was methylated with 1 ml ethereal diazomethane. After 10 min, the reagents were eliminated by evaporation (N_2 stream, 20°C). The resulting methyl ester was dissolved in 30 µl n-hexane and stored (−30°C) for the GC-MS analyses.

Results and Discussion

The advantage of using chemical ionization (either detecting positive or negative ions) over alternative techniques such as EI-GC-MS, GC-ECD, or GC-NPD for studying trace amounts of plant hormones in biological samples lies mainly in its increased sensitivity and selectivity, giving a much improved signal-to-noise ratio. In all the experiments reported here, the GC and ion source conditions employed were similar.

Qualitative Analyses

A mixture of equal amounts of IAA and ABA esters was injected to compare their behavior in the EI, PCI, and NCI modes. Three of the most frequently used reagent gases were employed—methane, isobutane, and ammonia. The resulting mass spectra indicate principally that it is possible to use the characteristic fragmentation patterns obtained with each of the reagent gases to con-

firm the identity of the compound (Table 1). For example, the $[M + 1]^+$ ion is important with the PCI mode using isobutane and ammonia. The $[M - 1]^-$ ion is typical of the IAA-Me molecule under NCI. For ABA-Me, the $[M - 17]^+$ and the $[M]^-$ are typical of the PCI and NCI modes, respectively. As for other molecules ionized under PCI conditions, ions at higher values than the molecular ions are recorded in the CI mode. This can be used to further the characterization of the compounds, especially for IAA (with M + 29 for methane and M + 17 for ammonia). For ABA, the loss of water seems from $[M + 1]^+$ to be an important feature in the PCI mode and from $[M]^-$ in the NCI mode. We also observed the quite significant difference in stability between the side chain of ABA-Me and that of t-ABA-Me under the various ionization modes tested, as has already been reported (Netting et al. 1982).

Quantitative Analyses

Several factors influence the choice for optimization of the signal intensities. In the present case, the source parameters were selected using perfluorotributylamine for EI and benzophenone for NCI with suitable reagent gas pressures. Basically, it is assumed that the efficiency of ionization of the compounds and the extent of their fragmentation influence the sensitivity of SIM detection. In Table 1, ion intensities, ionization, and fragmentation of base peaks are reported for IAA and ABA-methyl esters in the various ionization modes tested. The methane PCI mode gives the largest total ion current (TIC) for IAA-Me. However, the base peak at m/z 130 represents only 25% of the TIC. This indicates that monitoring such an ion will not allow a sensitive response to a low trace level of IAA-Me. On the other hand, the ammonia PCI mode produces a smaller TIC with much less fragmentation. Consequently, the m/z 190 SIM sensitivity will be better.

ABA has been reported to have electron-capturing properties without derivatization (Seeley and Powell 1974). It is thus not surprising that the ammonia NCI mode gives both the largest TIC and base peak responses. When the SIM parameters on four ions are optimized and only 1 ng of each compound is injected (corresponding to 0.01 of that used for the scanning mode), the response ratios calculated on the base peaks are as follows:

for IAA-Me	NCI/EI = 0.03	PCI/EI = 6.3
for ABA-Me	NCI/EI = 209	PCI/EI = 4.3
for t-ABA-Me	NCI/EI = 316	PCI/EI = 15

In order to increase the sensitivity, single ion detection was performed on the base peak, and the lower detection limit for ABA was determined. This is crucial when reaching the limit of sensitivity in the analysis of crude plant extracts. In the present case, m/z 278 with ammonia NCI was monitored, and increasingly diluted ABA-Me solutions were injected sequentially into the GC-MS (Fig. 1A). Once a signal was no longer obtained at maximum detector gain at the expected retention time (Fig. 1B), larger quantities were injected (Fig. 1C) and analyzed until a signal-to-noise ratio higher than 3 was obtained.

Table 1. Effect of the reagent gas on the ionization and the fragmentation of 100 ng each of IAA-Me and ABA-Me. Mass spectra are tabulated in Table 2 or have already been published (see text for references and mass spectra conditions).

Compound	Ionization	Mode	TIC area (counts)	Base peak m/z	Area	Percent of total ionization	Other ions[a]
IAA-Me	EI		5,443	130	2,144	39.4	189; 77; 131
	methane	positive	53,928	130	13,445	24.9	190; 189; 158
	methane	negative	10	188	—[b]		
	i-butane	positive	15,437	190	9,055	58.7	191; 189; 130
	i-butane	negative	3,000	188	342	15.3	—[b]
	ammonia	positive	48,001	190	22,612	47.1	207; 191; 130
	ammonia	negative	—[b]				
ABA-Me[c]	EI		2,345	190	275	11.7	125; 162; 134
	methane	positive	30,873	261	2,826	17.2	247; 203; 279
	methane	negative	27,037	278	14,002	51.8	260; 271; 262
	i-butane	positive	8,038	261	1,444	18.0	279; 263; 247
	i-butane	negative	61,413	278	27,140	44.2	260; 279; 297
	ammonia	positive	18,258	296	3,675	20.1	261; 279; 297
	ammonia	negative	57,871	278	35,640	53.6	260; 279; 260

[a] In decreasing abundance.

[b] Abundance too low to be measured.

[c] The mass spectra of trans-ABA differ from those of ABA just by the relative abundance of the same m/z values.

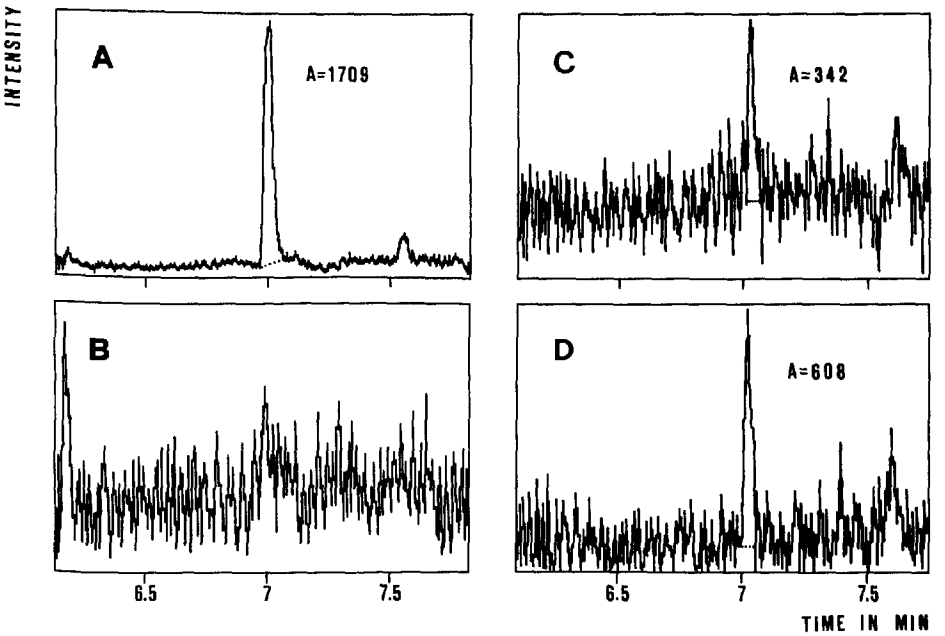


Fig. 1. Reconstructed SIM trace at m/z 278 using ammonia NCI. (A) Injection of 2 pg of ABA-Me followed by several injections of solvent until (B) no significant signal could be detected at the retention time of ABA-Me, after which (C) 0.1 pg of ABA-Me was injected giving a signal-to-noise ratio of 2. This was followed by (D) injection of 0.3 pg of ABA-Me giving a signal-to-noise ratio higher than 3. This is considered as the limit of the sensitivity of the setting.

This amount of ABA-Me injected was defined as the limit of detection (Fig. 1D) and was found to correspond to 0.3 pg. In this way, memory effect could be excluded. The value of 0.3 pg exceeds that obtained so far with ECD (Seeley and Powell 1974, Bittner and Even-Chen 1975), and with SIM the detection is based on a m/z value of significance. This good selectivity is illustrated for standards (Fig. 2) and extracts (Fig. 3) of *Zea mays* primary roots that were injected into the GCMS after esterification. Clearly, no quantitative measurements could be obtained with the EI mode. On the contrary, using ammonia NCI, precise peak area measurements for all four ions were possible.

Mono- and polyfluorinated derivatives of IAA are very volatile and have been used for sensitive ECD and SIM. In the present study, several volatile derivatives of IAA, which contained 1–10 fluorine atoms, have been synthesized (Fig. 4). Their mass spectra under different ionization modes have been recorded from 100 ng injections (Table 2). The yield of the derivatization was estimated to be higher than 95% in each case, as shown by the IAA-Me detected by GC when the reaction mixture was evaporated and esterified with diazomethane. Semiquantitative data were then obtained from the mass spectra, and the respective merits of each derivative relative to GC behavior and MS ionization mode were compared (Table 3).

From the various derivatives tested here, all except 5-F-IAA-Me have been

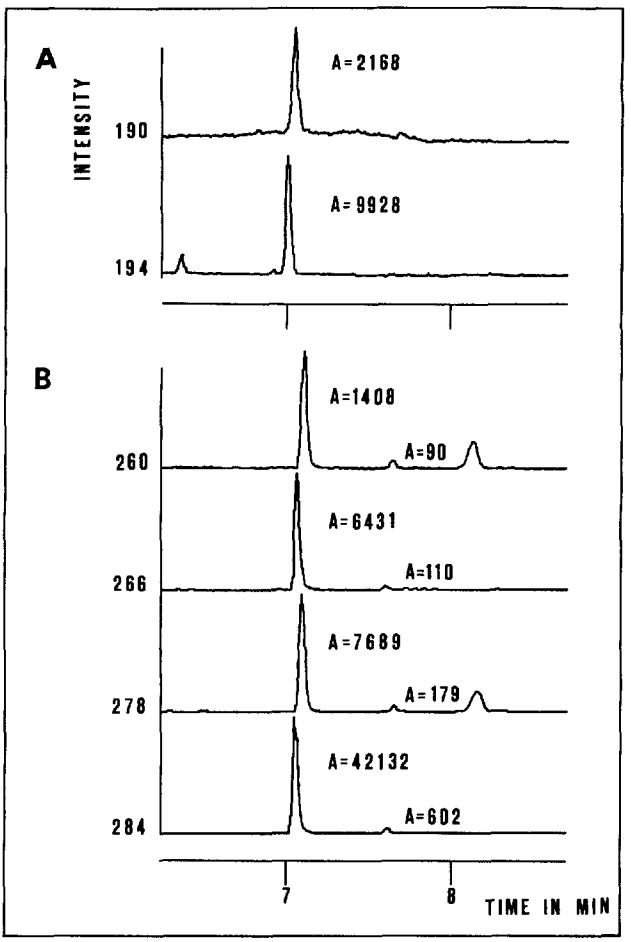


Fig. 2. Reconstructed selected ion traces of ABA standards: 5 ng ABA and 20 ng hexadeuterated-ABA were injected and detected under (A) EI conditions where m/z 190 is the peak of ABA-Me and m/z 194 that of hexadeuterated-ABA-Me, and (B) ammonia NCI conditions where m/z 278 and 284 are the base peak masses of ABA-Me and hexadeuterated-ABA-Me, respectively; m/z 260 and 266 are the masses of the first fragmentation peak. The second peak of each channel is that of the t-ABA derivative.

used for EI-SIM determinations in plant extracts (Davis et al. 1968, Rivier and Pilet 1974, Caruso et al. 1978, Little et al. 1978, McDougall and Hillman 1978, Allen and Baker 1980, Iino et al. 1980, Epstein and Cohen 1981). IAA-Me gives very little negative-ion response, as was also the case for IAA-Et (Epstein and Cohen 1981). For PCI, methane is the reagent of choice, as it offers both excellent sensitivity and some fragmentation useful for confirming peak identity and purity. However, ammonia is better when two ions are monitored, as this combination gives major ions at m/z 190 for $[M + 1]^+$ and m/z 207 for $[M + 18]^+$.

The IAA-Me-HFB derivative offers favorable fragmentation under EI, giving two important ions at relatively high mass values. PCI is, however, even more favorable. Surprisingly, the best sensitivity under PCI is obtained when one fluorine atom is attached to the IAA molecule. The compound tested was not obtained directly from IAA, and attempts to introduce a single fluorine atom at other positions were not tried here. No attempts using greater amount of IAA were made, as its trihalogenated ethyl esters are known to produce a

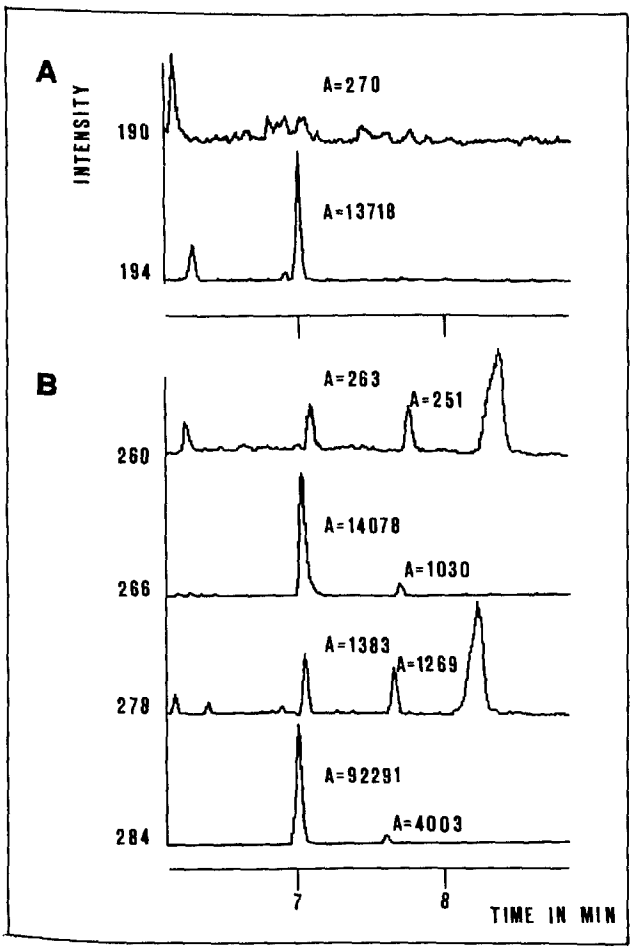
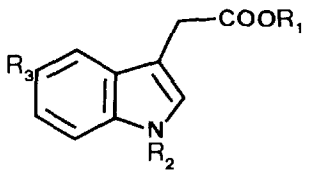


Fig. 3. Reconstructed selected ion traces of methylated extracts of *Zea mays* root segments to which 20 ng hexadeuterated ABA has been added at the beginning of the procedure. The injections were done under (A) EI-GC-MS conditions and (B) ammonia NCI conditions, and the m/z are the same as in Fig. 2.

weaker electron capture response than the PFP derivatives (Bittner and Even-Chen 1975). The readily prepared PFP derivative of IAA when 10 fluorine atoms are added shows outstanding volatility and the highest response when NCI was used with ammonia. This derivative has been tested on plant extracts in the EI mode (Allen and Baker 1980). IAA-TMS and IAA-PFB were also included in the comparison, as has been recommended (McDougall and Hillman 1978, 1980, Epstein and Cohen 1981).

To summarize, the IAA molecule, whatever the derivative obtained so far, is not at all suitable for NCI. Better results are clearly obtained with PCI on IAA-Me or with EI on IAA-Me-HFB. On the other hand, NCI can be used with great improvement of the signal-to-noise ratio for ABA-Me. Almost ideal fragmentation is obtained, and improved sensitivity is achieved compared to the traditionally used EI mode or indeed the electron-capture detection. Endogenous IAA has been quantified by GCMS, using the IAA-Me-HFB derivative in EI mode, in growing maize (cv. LG 11) roots (Pilet and Saugy 1985). It was of interest to quantify the ABA in the roots of the same variety using the



Name	R_1	R_2	R_3
Indol-3yl-acetic acid (IAA)	H	H	H
Indol-3yl-acetic acid methylester (IAA-Me)	CH_3	H	H
Indol-3yl-acetic acid bis trimethyl silyl derivative (IAA-TMS)	$(CH_3)_3Si$	$(CH_3)_3Si$	H
5-Fluoro-indol-3yl acetic acid methyl ester (5-F-IAA-Me)	CH_3	H	F
Indol-3yl-acetic acid trifluoro-acetyl-1 (IAA-Me-TFA)	CH_3	F_3CCO	H
Indol-3yl-acetic acid pentafluoro-benzylester (IAA-PFB)	$F_5C_6CH_2$	H	H
Indol-3yl-acetic acid methylester heptafluorobutanoyl-1 (IAA-Me-HFB)	CH_3	F_7C_3CO	H
Indol-3yl-acetic acid pentafluoro-propionylester pentafluoropropionyl-1 (IAA-PFP)	$F_5C_2CH_2$	F_5C_2CO	H

Fig. 4. Formulae of the IAA derivatives tested for PCI and NCI.

best technique of detection of ABA described above: NCI with ammonia as the reagent gas on the ABA-Me derivative. This technique allowed us to collect results from as little as 40–50 mg of fresh weight plant material. Furthermore, the extraction procedure is short enough to minimize the losses which are a problem when small amounts have to be used as the starting material.

ABA Level along the Root

The level of ABA (expressed per sample, per gram of fresh weight, and per gram of dry weight) for different regions between 0 and 15 mm (measured from the tip) of the maize root is shown in Table 4. ABA can be found all along the 15-mm-long root tip with the highest concentration in the differentiated tissues. This gradient could be explained by the basipetal transport of ABA from the cap (Pilet 1976) producing an accumulation in the differentiated part of the root. These data differ from previous results found with *Pisum* (Böttger 1978) and another cultivar of maize (Rivier et al. 1977). Rivier and Pilet (1981) showed that several varieties of maize exhibited very different levels of ABA in the root tip. Nevertheless, it appeared that the different regions of the root contained different amounts of ABA, and this has to be kept in mind in the second experiment. In this, only the root tip (0–2.5 mm) and the growing zone were collected separately. We postulated that the differentiated tissues are not involved in the growth. Consequently, we collected the parts directly related to the growth. For technical reasons, it was impossible for us to separate the cap and the apex in the root tip sections (0–2.5 mm).

Table 2. Tabulation of mass spectra of various derivatives of IAA in the EI, PCI, and NCI modes.

Compound	Mode	Base peak	2nd peak[a]	3rd peak[a]	4th peak[a]	5th peak[a]
Indolyl-3-acetic acid methyl ester: IAA-Me						
	EI	130	189 (31)	77 (15)	131 (10)	190 (8)
ammonia	PCI	190	207 (61)	191 (13)	130 (10)	208 (7)
	NCI	188	189 (15)	175 (3)	161 (2)	174 (1)
methane	PCI	130	190 (84)	189 (20)	158 (18)	218 (11)
	NCI	188				
i-butane	PCI	190	191 (12)	189 (8)	130 (5)	228 (3)
	NCI	188	150 (67)	194 (60)	162 (50)	178 (48)
Bis-trimethylsilyl-indolyl-3-acetic acid: IAA-TMS						
	EI	202				
ammonia	PCI	321	322 (25)	392 (16)	323 (7)	393 (4)
	NCI	319	389 (12)	299 (11)	289 (9)	213 (8)
5-Fluoro-indolyl-3-acetic-acid: 5-F-IAA-Me						
	EI	148				
ammonia	PCI	226	209 (79)	227 (13)	219 (10)	208 (6)
	NCI	206	207 (14)	192 (1)	176 (1)	162 (1)
N-trifluoroacetyl-indolyl-3-acetic acid methyl ester: IAA-Me-TFA						
	EI	226				
ammonia	PCI	304	305 (16)	286 (10)	227 (4)	191 (2)
	NCI	285	284 (43)	265 (30)	286 (12)	187 (8)
N-heptafluorobutyryl-indolyl-3-acetic acid methyl ester: IAA-Me-HFB						
	EI	326				
ammonia	PCI	403	404 (11)	385 (8)	208 (3)	327 (2)
	NCI	384	365 (98)	347 (32)	245 (24)	385 (19)
Indolyl-3-acetic acid pentafluoropropionyl ester pentafluoropropionyl-I: IAA-PFP						
	EI	276				
ammonia	PCI	437	326 (82)	309 (50)	453 (28)	420 (14)
	NCI	305	433 (26)	308 (12)	307 (9)	452 (1)

[a] m/z of the peak with its abundance in parentheses expressed in percent of the abundance of the base peak.

Table 3. Chromatographic behavior and yield of ionization and fragmentation of fluorinated derivatives of IAA: factors of choice for SIM determinations. Ammonia was used for CI.

				Base peak (area response) % of total ionization		
				Ionization mode		
Compound	Number of F atoms	Rt[a] (min)	MW[b]	EI	PCI	NCI
IAA-Me	0	7.00	189	130 (2,144)39	190 (22,612)47	188 (2,161)44
5-F-IAA-Me	1	6.40	207	148 (6,525)43	226 (48,570)46	206 (9,228)73
IAA-Me-TFA	3	5.57	285	226 (2,209)39	304 (10,339)58	285 (7,655)44
IAA-PFB	5	9.67	355	130 (528)32	303 (1,420)36	335 (221)20
IAA-Me-HFB	7	5.62	385	326 (7,371)44	403 (25,820)65	384 (118)33
IAA-PFP	10	5.12	453	276 (7,655)46	437 (2,036)22	305 (10,768)62

[a] Retention time obtained on a cross-linked fused silica capillary column of the SE-54 type with temperature programming as described in the text.
[b] Molecular weight.
The area response is the area of the peak calculated on the SIM chromatogram.

Table 4. Endogenous ABA level in different parts of maize roots.

Root section (mm)		Number of segments per sample	ng ABA per sample	ng ABA per g FW	ng ABA per g DW
C	0.0–0.5	100	0.33 ± 0.03	7.5 ± 1.0	49.8 ± 9.1
AP	0.5–1.0	100	0.50 ± 0.04	8.6 ± 1.1	46.3 ± 5.8
IZ	1.0–2.5	50	1.36 ± 0.10	17.2 ± 1.5	92.5 ± 10.8
EZ	2.5–5.0	50	2.95 ± 0.10	21.9 ± 1.2	180.8 ± 12.0
DZ I	5.0–10.0	30	5.78 ± 0.30	33.8 ± 2.4	325.7 ± 29.1
DZ II	10.0–15.0	30	7.48 ± 0.46	36.3 ± 2.7	365.7 ± 26.8

Intact 48-h-old seedlings with 16 ± 0.5 mm long roots were used in this experiment.
C, cap; AP, apex; IZ, intermediate zone; EZ, elongation zone; DZ I, first part of differentiation zone; DZ II, second part of differentiation zone.

Table 5. Growth rate, fresh weight, and dry weight of the root tip and the elongation zone from different maize root growth classes.

Growth classes (No. segments)	Growth rate (mm h^{-1})	Root tip per 30 segments		Elongation zone per 30 segments	
		FW (mg)	DW (mg)	FW (mg)	DW (mg)
I (60)	0.243 ± 0.011	44.1 ± 3.3	16.8 ± 1.6	66.7 ± 1.0	17.5 ± 2.4
II (90)	0.359 ± 0.003	43.6 ± 1.2	13.8 ± 0.5	63.4 ± 2.5	13.8 ± 0.2
III (90)	0.428 ± 0.001	42.1 ± 0.8	13.8 ± 0.8	62.2 ± 0.4	12.4 ± 0.7
IV (150)	0.501 ± 0.002	49.2 ± 1.9	15.1 ± 0.7	64.2 ± 1.3	12.7 ± 0.1
V (120)	0.602 ± 0.003	44.2 ± 1.2	13.5 ± 0.4	62.0 ± 2.4	11.7 ± 0.9
VI (90)	0.702 ± 0.003	42.1 ± 0.2	12.3 ± 0.5	59.7 ± 1.8	10.9 ± 1.1
VII (90)	0.803 ± 0.004	42.3 ± 0.7	12.0 ± 0.3	57.1 ± 1.2	10.5 ± 0.4
VIII (90)	0.932 ± 0.004	45.9 ± 1.5	11.5 ± 0.3	66.4 ± 2.6	10.6 ± 0.3
IX (60)	1.091 ± 0.090	41.0 ± 0.5	12.0 ± 0.5	58.1 ± 4.3	10.0 ± 0.7
Total (840)	0.603 ± 0.050	43.8 ± 0.8	13.4 ± 0.6	62.2 ± 1.1	12.2 ± 0.8
Control (240)	0.860 ± 0.011	55.8 ± 2.0	11.7 ± 0.7	77.6 ± 0.8	10.5 ± 0.7

Intact seedlings with 16 ± 0.5 mm long roots were maintained vertically in the dark for 8 h. Each segment was then prepared and stored individually before being attributed to a growth class. Thirty segments were used for each assay; the control group represents the class of segments from roots at the beginning of the experiment.

ABA Level and Growth Rate

The variation in the growth rate (expressed in mm/h) of 2-day-old maize roots kept vertical and attached to their caryopses was given in the previous work (Pilet and Saugy 1985), involving the relation between growth and IAA. The great sensitivity of the present method of detection of ABA allowed us to use a smaller number of roots for each determination than for IAA detection. Therefore the whole population was divided into nine classes, and each sample was composed of 30 segments (root tips or elongation zone segments). These classes are characterized (Table 5) by the mean of the root growth, the fresh weight, and the dry weight for 30 segments. No significant differences were noticed between the growth classes from data calculated on the fresh and dry weight basis. But the control group, which was composed of segments from roots at the beginning of the experiment, had a higher fresh weight than the other roots tested.

Endogenous ABA content of the segments from the nine growth classes is reported in Fig. 5. The results, obtained by calculations based on the molecular ion peak, are expressed in nanograms of IAA per 30 segments. The data described in Fig. 5 showed clearly that the higher the ABA level in the root, the lower the root growth rate. In class II, characterized by a growth rate of 0.359 mm h^{-1}, the ABA level was 10.17 ng per 30 growing zones, whereas in class VIII, whose growth rate was 0.952 mm h^{-1}, the ABA level was 2.33 ng per 30 segments. The shape of the curve indicates that a small difference in the growth of the slowly growing roots corresponds to a large change in the ABA level and that, on the other hand, rapidly growing roots all contain relatively small amounts of ABA. This attests to the variability of the endogenous ABA level in plant material; therefore, in the study of physiological properties of

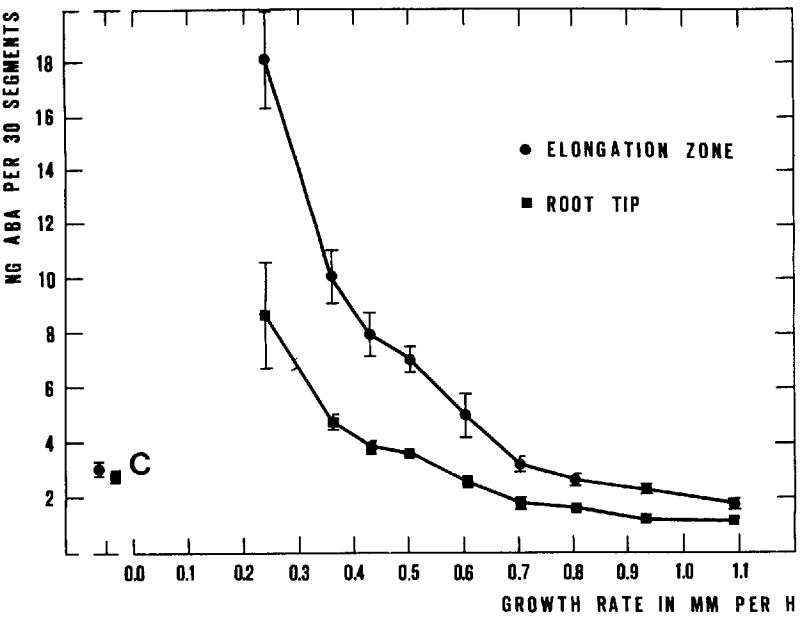


Fig. 5. Variation in the endogenous ABA level (in ng ABA ± SE per 30 segments) as a function of the growth rate (in mm ± SE per h). ABA levels are given for the root tip (0–2.5 mm) and the elongation zone (2.5–5 mm) segments. The control (C) represents the endogenous ABA level of segments at the beginning of the experiment. For the number of determinations and the technique of preparation of the material, see Table 5.

roots through gravitropism or light effects, great attention to these results should be paid. Furthermore, this relation is confirmed by the observation of the control roots. Their mean growth rate (0.86 mm h^{-1}) at the beginning of the experiment was higher than that of the same roots that had been inside the boxes for 8 h (0.60 mm h^{-1}). This difference is due to the experimental manipulations and is most likely the consequence of water deficiency. Before the experiment, the seedlings were growing between two moist filter papers (Pilet 1977). Technically, this setting was not possible for the 8-h experiments. The fresh weight of the roots (Table 5) was significantly lower after 8 h in the boxes than at the beginning of the experiment (control). The ABA level of the control group that corresponded (Fig. 5) to that of the rapid-growth classes assumes that such a relation exists under different conditions. It is therefore important to choose experimental conditions that take these observations into account.

In conclusion, the use of a new specific and highly sensitive method of quantification and the establishment of growth classes allowed us to demonstrate a clear relation between endogenous ABA level and growth. Additional experiments will be necessary to find the localization in the tissue of the regulation of ABA level in relation to physiological events.

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