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Jasmonic Acid and Abscisic Acid in Shoots, Coleoptiles, and Roots of Wheat Seedlings

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Abstract. The endogenous levels of abscisic acid (ABA) and jasmonic acid (JA) were analyzed in wheat seedlings grown in water, a system which in the past has been used to test the effects of these plant growth inhibitors. The levels in different plant parts and in the medium were measured by gas chromatography-mass spectrometry-selected ion monitoring, using [$^2H_3$]ABA and [$^2H_6$]JA as internal standards. In every plant part, JA levels were about 2 orders of magnitude greater than those of ABA. The exudation of JA from roots per seedling was about 14,000-fold greater than that of ABA, although the roots contained only about 170 times more JA than ABA. It is suggested that JA is a possible allelopathic compound.

The natural growth inhibitor jasmonic acid (JA) and many of its analogues with sterical and structural modifications of the side chains and the cyclopentanone ring occur throughout the plant kingdom (Meyer et al. 1984; Sembdner et al. 1989; Sembdner and Parthier 1993). Some of these compounds appear to be involved in senescence regulation and stress responses (Parthier 1991).

Wheat seedling growth has been used as a bioassay for the detection of JA in *Vicia faba* (Dathe et al. 1981). Detailed analysis by Dathe (1988, 1992) revealed significant differences in the effects in this bioassay of JA and its methyl ester (JAMe) and of ABA, applied *via* the roots. ABA inhibited the growth of the first leaf much more effectively than did JA or JAMe. Moreover, ABA inhibited both leaf blade and leaf sheath growth, whereas JA inhibited mainly the leaf blade growth. On the other hand, root growth was more sensitive to inhibition by JAMe than by ABA (Dathe 1992). The analysis in the present paper of the endogenous levels of JA and ABA in wheat seedlings, and their changes during development, was undertaken to provide further information on the roles of these growth regulators.

Materials and Methods

Plant Material

Seeds of spring wheat (*Triticum aestivum* L. cv. 'Hatri') were sown on moist filter paper and allowed to germinate over 2 days in darkness at 22°C. Uniform seedlings (coleoptile length 3–5 mm, three primary roots) were transferred to a growth vessel containing distilled water (100 seedlings/100 ml) and cultivated for an additional 3 or 5 days at 20°C under a 16-h photoperiod (6500 lux). The pH of the culture medium shifted from 5.15 (day 0) to 4.87 ± 0.07 after 3 days and to 4.44 ± 0.09 after 5 days. At harvest, the seedlings were divided into leaves, coleoptiles, and roots. The plant materials from each part were blotted briefly on filter paper, frozen with liquid nitrogen, and stored at −20°C. The water in which the seedlings had grown was also stored. Further characteristics of the plant material are given in Table 1. Culture conditions were not sterile, as production of ABA or JA by microorganisms is believed to be rare.

Extraction, Purification, and Quantification of JA and ABA

The seedling parts were homogenized in a mortar (coleoptiles) or in a Waring Blender (leaves, roots) and extracted with methanol and then twice with 80% aqueous methanol. The following internal standards were added: [$^2H_3$]ABA (Neill and Horgan 1987) in 30- to 150-ng amounts depending on the sample, and [$^2H_6$]JA

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Table 1. Some characteristics of the plant materials

Plant materials	Treatment (days)	Length (cm)	Weight (mg/part)
Leaf	5	6.2 ± 0.8	19.0 ± 2.4
	7	10.5 ± 1.5	41.1 ± 3.5
Coleoptile	5	3.4 ± 0.2	21.4 ± 2.0
	7	3.4 ± 0.2	19.8 ± 1.7
Root	5		30.7 ± 3.2
	7		49.2 ± 4.1

Note: The seeds germinated during 2 days, the young uniform seedlings were transferred into water, cultivated for 3 or 5 days therein, and then harvested at an age of 5 or 7 days, respectively. Numbers of seedlings are 614 (5 d) and 531 (7 d).

(Miersch 1991) in 10- to 50-μg amounts to the combined extract solutions. The methanolic extracts with internal standards were then evaporated to the aqueous phase, frozen, thawed, centrifuged, adjusted to pH 3.0, and partitioned three times with chloroform. The culture media were mixed with 500 ng [$^2H_3$]ABA and 15–50 μg [$^2H_6$]JA and partitioned with chloroform in the same way.

The organic phases, after evaporation, were purified by column chromatography on DEAE-Sephadex A-25 using a continuous gradient of 80% acetic acid in methanol (Gräbner et al. 1976). Fractions corresponding to JA and ABA (eluted with 0.25–0.5 M acetic acid) were combined, evaporated and loaded onto a 400-mg cartridge of Adsorbex RP-18 (Merck, Darmstadt, Germany). The elution was performed with an increasing gradient of methanol in 0.2% acetic acid. The fractions corresponding to JA and ABA (eluted with 40%–60% methanol) were evaporated, methylated with diazomethane (Neill and Horgan 1987) and analyzed by gas chromatography-mass spectrometry (GC/MS) on a Kratos MS25 mass spectrometer coupled with a Carlo Erba 4200 gas chromatograph (Kratos Ltd, Manchester, UK).

GC was performed on a 15 m × 0.32 mm Mega-OV1 column (Carlo Erba Instruments, Crawley, UK), film thickness 45 μm, He as carrier gas (0.3 kp · cm^{-2}), with a temperature program of 45°–180°C (ballistic) to 260°C (at 8°C · min^{-1}). The GC/MS interface was at 200°C, the source at 190°C, the ionizing voltage was 70 eV, and the resolution was ≥600. Selected ion monitoring (SIM) was performed with a dwell time of 200 msec and a setting time of 100 msec. The ions monitored were m/z 224.1 (JAMe) and 230.2 ([$^2H_6$]JAMe) at R_t = 2.34 – 2.39 min and 190.1 (ABAMe) and 193.1 ([$^2H_3$]ABAMe) at R_t = 6.35 – 6.41 min.

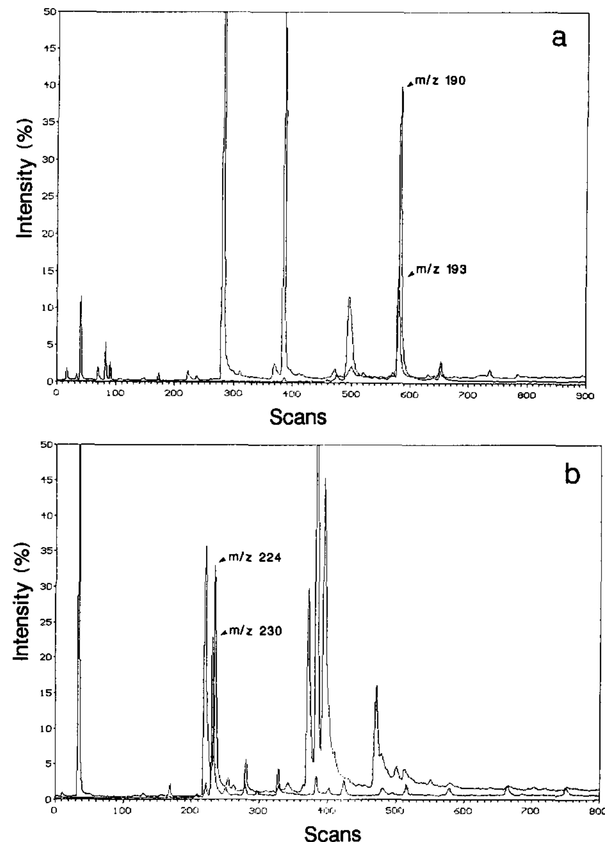

Fig. 1. Mass spectrometric quantification of **(a)** ABA and **(b)** JA in wheat leaves 5 days after germination. Both compounds were derivatized to their methyl ester. The SIM chromatograms shown were obtained by accelerating voltage switching to detect **(a)** m/z 190 for ABAMe and m/z 193 for [$^2H_3$]ABAMe; and **(b)** m/z 224 for JAMe and m/z 230 for [$^2H_6$]JAMe.

Results

Abscisic acid was measured by GC/MS selected ion monitoring using a trideuterated internal standard (Fig. 1a). The first leaf of 5-day-old wheat seedlings was found to contain more ABA per gram of tissue than did the coleoptile or root (Table 2). Over the next 2 days of growth, the leaf ABA content increased further. ABA levels remained nearly constant in the coleoptile (which had reached maximum length), and declined slightly in the roots (Table 2).

The levels of JA were likewise measured by GC/MS, using a hexadeuterated internal standard (Fig. 1b), and were found to be generally more than 100 times higher than those of ABA. The total JA content of the first leaf increased between days 5 and 7, although as this leaf expanded the JA content per gram of tissue decreased (Table 3). During this period, the JA content per organ of the coleoptile and root remained nearly constant, although in the root the JA content per gram declined as this organ expanded.

Measurements of the ABA and JA in the aqueous growth medium revealed a dramatic difference in the exudation of the two compounds from the roots (Table 4). After 7 days, the amount of ABA in the medium per seedling was about 0.8 of the mean ABA content of a single root system. However, the amount of JA in the medium per seedling at this time was 65 times the mean JA content of a single root system.

The measurements were replicated on a further batch of seedlings with similar results.

Table 2. The levels of ABA in different parts of wheat seedlings

	Age of seedlings (days)			
	5		7	
Material	Concentration (ng/g fr. wt.)	Content (ng/part)	Concentration (ng/g fr. wt.)	Content (ng/part)
Leaf	3.7	0.071	6.9	0.282
Coleoptile	1.4	0.030	1.4	0.028
Root	2.0	0.061	1.0	0.047

Note: The levels were determined by GC-MS-SIM using [$^2H_3$]ABA as internal standard.

Table 3. The levels of JA in different parts of wheat seedlings

	Age of seedlings (days)			
	5		7	
Material	Concentration (ng/g fr. wt.)	Content (ng/part)	Concentration (ng/g fr. wt.)	Content (ng/part)
Leaf	1012	19	588	24
Coleoptile	661	14	703	14
Root	245	7.5	162	8.0

Note: The levels were determined by GC-MS-SIM using [$^2H_6$]JA as internal standard.

Table 4. The levels of ABA and JA in the aqueous solution

	Age of seedlings (days)	
Substance	5	7
ABA	0.018	0.038
JA	383	523

Note: The seedlings grew during 3 (5-day-old seedlings) or 5 days (7-day-old seedlings), respectively. The values are given in ng JA or ABA per seedling grown in the solution. The levels were determined by GC-MS-SIM using [$^2H_3$]ABA and [$^2H_6$]JA as internal standards.

Discussion

The JA content of each of the wheat seedling parts was generally about two orders of magnitude higher than their ABA content. The measurements of the endogenous levels of these two growth inhibitors can be examined in relation to previous studies (Dathe 1988, 1992) of their action and metabolism as exogenous treatments. The elongation of the first leaf of wheat is about 100–200 times more effectively inhibited by ABA than by JA (applied *via* the roots) and the elongation of the coleoptile is likewise inhibited about 50–100 times more effectively by ABA applied in this way. JA and ABA are equally effective in the inhibition of wheat root growth, whereas JAMe is more effective than ABA (Dathe 1988; Dathe et al. 1993). Therefore, when the high levels of JA in the tissue are taken into consideration, it would appear that this compound could play a physiological regulatory role.

Dathe (1992) found that JA was taken up and metabolized by wheat roots, but did not thence enter the shoots. This result suggests that the inhibition of shoot organs by JA applied *via* the roots could be a result of effects in the roots. Another factor to be considered is that the high exudation of JA from the roots might be affected by the exogenous JA in the culture medium, in turn affecting the endogenous levels of JA. The morphology and permeability of roots are significantly altered by JA (Ravnikar et al. 1990; Vilhar et al. 1991) and therefore it can be assumed that JA treatment results in a complex interaction involving membrane permeability and metabolism in roots.

The much greater exudation of JA than of ABA is probably due to the membrane permeability of the former compound being two orders of magnitude greater (Dathe et al. 1993). It is possible that JA or derivatives exuded from roots have an allelopathic effect, either on neighboring plants or on the rhizosphere microorganisms; JA inhibits the growth of mycorrhizae at only 10^{-10} M (Gogala 1987). Methyl jasmonate as a volatile compound emitted by leaves is involved in interplant communication, inducing

proteinase inhibitor gene expression in neighboring plants (Farmer and Ryan 1990).

ABA levels in soil-grown wheat seedlings have been reported to be about one order of magnitude higher than those in the water-grown seedlings used in the present study, perhaps reflecting the responsiveness of this hormone to environmental conditions, or the different varieties used (Buta and Spaulding 1991).

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