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Jasmonic acid distribution and action in plants: Regulation during development and response to biotic and abiotic stress

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ABSTRACT Jasmonic acid (JA) is a naturally occurring growth regulator found in higher plants. Several physiological roles have been described for this compound (or a related compound, methyl jasmonate) during plant development and in response to biotic and abiotic stress. To accurately determine JA levels in plant tissue, we have synthesized JA containing ^{13}C for use as an internal standard with an isotopic composition of [225]:[224] 0.98:0.02 compared with [225]:[224] 0.15:0.85 for natural material. GC analysis (flame ionization detection and MS) indicate that the internal standard is composed of 92% 2-(±)-[^{13}C]JA and 8% 2-(±)-7-iso-[^{13}C]JA. In soybean plants, JA levels were highest in young leaves, flowers, and fruit (highest in the pericarp). In soybean seeds and seedlings, JA levels were highest in the youngest organs including the hypocotyl hook, plumule, and 12-h axis. In soybean leaves that had been dehydrated to cause a 15% decrease in fresh weight, JA levels increased \approx 5-fold within 2 h and declined to approximately control levels by 4 h. In contrast, a lag time of 1–2 h occurred before abscisic acid accumulation reached a maximum. These results will be discussed in the context of multiple pathways for JA biosynthesis and the role of JA in plant development and responses to environmental signals.

Jasmonic acid (JA) and its methyl ester, methyl jasmonate (JAMe), are naturally occurring regulators of higher plant development, responses to external stimuli, and gene expression (Fig. 1) (for reviews, see refs. 1–4). JA was first isolated from cultures of the fungus *Lasiodiplodia theobromae* (5). However, most of the initial interest was in JAMe because of its volatility and presence in essential oils of *Jasminum grandiflorum* L. and *Rosmarinus officinalis* L. (6, 7). Commercial interest in JAMe by the perfume industry stimulated the study of its structure and synthesis (2). Numerous derivatives of JA are found in plants including hydroxylated forms such as tuberonic acid and cucurbitic acid and amino acid conjugates (2). The role of most of the derivatives of JA is unclear although tuberonic acid has been proposed to regulate tuber formation in potato (for review, see ref. 4). JA has been found in all higher plants examined and in general is estimated to be present at concentrations $<10\ \mu\text{M}$ (8). The level of JA in plant tissues varies as a function of tissue type, development, and external stimuli (for reviews, see refs. 1–4). The highest levels of JA/JAMe are reported in flowers and reproductive tissues, whereas much lower levels are found in roots and mature leaves. Jasmonate can move readily in plants in the liquid and vapor phase (i.e., ref. 9). Changes in plant gene expression are induced by nanomolar to micromolar concentrations of JA/JAMe.

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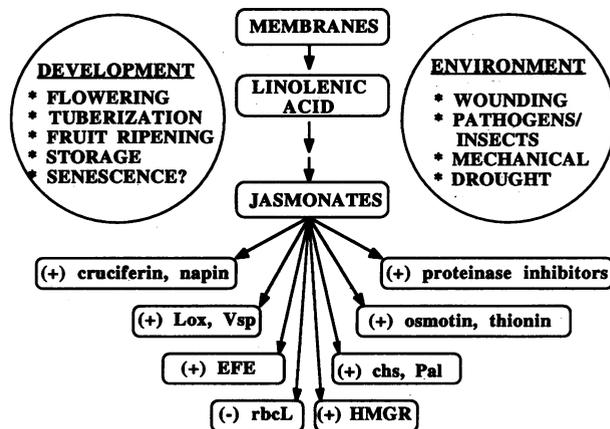


Fig. 1. Diagram showing synthesis of JA from linolenic acid in response to developmental and environmental signals. Genes that are induced by jasmonates are listed (*Vsp*, vegetative storage protein acid phosphatases; *Lox*, lipoxygenase; *EFE*, ethylene forming enzyme; *rbcL*, large subunit of ribulose biphosphate carboxylase; *pinII*, proteinase inhibitor II; thionin, antifungal protein; osmotin, antifungal protein; *chs*, chalcone synthase; *Pal*, phenylalanine ammonia lyase; HMGR, hydroxymethylglutaryl CoA reductase).

Jasmonates are derived from linolenic acid in a lipoxygenase-dependent process (Fig. 1) (for reviews, see refs. 2 and 3). Their structure is similar to mammalian eicosanoids, which are also derived from lipids through the action of lipoxygenase (2). Early work on the JA biosynthetic pathway by Vick and Zimmerman (3) indicated that linolenic acid could be converted into the cyclopentanone 12-oxophytodienoic acid (12-oxo-PDA). This conversion is now known to involve lipoxygenase, which mediates peroxidation of linolenic acid, followed by the action of allene oxide synthase and allene oxide cyclase (for review, see ref. 2). Allene oxide synthase has been purified and is a 55-kDa cytochrome P450 enzyme (10). The resulting 12-oxo-PDA is converted into JA through reduction and three cycles of β -oxidation (2). Allene oxide cyclase could be a key step in this pathway because the substrate for this enzyme can be converted into ketols instead of JA (2). Although the biosynthetic pathway of JA is now clear, there is less information concerning how JA biosynthesis is regulated during plant development or in response to biotic and abiotic stress.

JA can influence several aspects of plant growth and development (Fig. 1). Jasmonate can induce senescence and leaf abscission and inhibit germination (1–4). At concentrations

Abbreviations: JA, jasmonic acid [the natural enantiomer (–)-jasmonic acid unless defined otherwise]; JAMe, methyl (–)-jasmonate; 12-oxo-PDA, 12-oxophytodienoic acid; ABA, abscisic acid. *To whom reprint requests should be addressed.

>50 μM , jasmonate induces senescence in plant cell cultures and excised leaves. The senescence response includes a loss of chlorophyll, degradation of chloroplast proteins such as ribulose biphosphate carboxylase, and accumulation of new proteins. Jasmonate specifically inhibits translation of the large subunit of ribulose biphosphate carboxylase by inducing cleavage of the *rbcL* transcript (11). While jasmonate can induce senescence when applied at high concentrations, it is not clear whether this regulator modulates senescence *in vitro*. To our knowledge, the level of jasmonate in senescing leaves has not been reported. Moreover, the highest levels of jasmonate accumulate in actively growing tissues such as hypocotyl hooks, flowers, and developing seed pods. JA inhibits root growth (12) and induces tuber formation (4). Jasmonate can also induce tendril coiling (13). Tendril coiling is induced by mechanical stimulation and roles for auxin and ethylene have been proposed. Although jasmonate can stimulate ethylene biosynthesis by inducing activity of the ethylene forming enzyme (14), studies on tendril coiling showed that jasmonate can mediate this response in the presence of an inhibitor of ethylene biosynthesis (13). This study also demonstrated a correlation among mechanical stimulation, reduction in specific classes of lipids that could be precursors of jasmonate, and the induction of jasmonate (13). Jasmonate has been shown to stimulate fruit ripening, most likely through its action on ethylene biosynthesis (14). In addition, jasmonate stimulates the conversion of lycopene to β -carotene (15).

A role for JA in plant response to water deficit has been suggested because this stress induces the expression of several genes that also respond to JA (refs. 16 and 17; for review, see ref. 1). For example, expression of the genes encoding the soybean vegetative storage protein acid phosphatases (*VspA/VspB*) was increased in plants subjected to water deficit and in plants treated with JA (16, 18). *VSP α* and *VSP β* accumulate in soybean vacuoles and to a lesser extent in cell walls. Based on their abundance, localization, and accumulation in depodded plants the *VSP* acid phosphatases were identified as vegetative storage proteins (for review, see ref. 19). *VSP* mRNA levels increase in wounded tissue due to the accumulation of JA (20). Analysis of the *VspB* promoter revealed the presence of a DNA domain that mediates responses to JA (21). In addition, a second DNA domain was found to mediate responses to sugars (positive) (21), auxin (negative) (22), and phosphate (negative) (23). One question the present study addresses is whether JA increases in plant tissues exposed to water deficit.

In addition to its role in plant growth and development, jasmonate has been proposed as a key regulator of plant responses to pathogens and insects (Fig. 1; for review, see ref. 24). This role was suggested when low concentrations of jasmonate were found to induce genes encoding proteinase inhibitors (9), enzymes involved in flavonoid biosynthesis (chalcone synthase and phenylalanine ammonia lyase) (20, 25), sesquiterpenoid biosynthesis (hydroxymethylglutaryl CoA reductase) (26), thionin (antifungal protein) (27), and osmotin (antifungal protein) (28). Jasmonate also induces expression of lipoxygenase (17). Lipoxygenase is involved in the biosynthesis of jasmonate and has been implicated in plant responses to pathogens (29). The induction of lipoxygenase may make the plant more sensitive to further pathogen attack or enhance the plants' capacity to synthesize other lipid-derived compounds used in plant defense. The role of JA in plant defense was further supported by the finding that wounding (20) and pathogen elicitors (25) induce accumulation of JA.

The role of JA could be clarified by accurate and precise determination of its levels under known physiological conditions. Several methods have been used to estimate JA levels in plant tissues. Initially, quantitation was performed by using bioassays based on JA's inhibitory properties (senescence promotion and seedling growth inhibition) (30). Radioimmunoassay has also been used to determine JA levels (31).

Anderson (32) described an HPLC assay based on the coupling of JA with a fluorescent hydrazide giving a stable fluorescent product. All of these methods, however, are limited in utility due to a lack of specificity, sensitivity, or inability to estimate recovery. Weiler *et al.* (13) measured JA levels by addition of [^3H]JAMe to partially purified methylated plant extracts followed by further HPLC purification and analysis by ELISA. Sample recovery prior to the addition of [^3H]JAMe was not determined. In general, the best method for plant hormone quantitation is to use heavy isotope-labeled internal standards coupled with GC/MS. By using 9,10-dihydro-JA as an internal standard, Gundlach *et al.* (25) measured JA in suspension cultures. Creelman *et al.* (20) used the recovery of [$^2\text{H}_3$, ^{13}C]JAMe to estimate recovery of JA. A homolog, however, can have slightly different solvent partition characteristics and may not be recovered to the same degree as the compound of interest.

In this paper, we have synthesized (\pm)-[^{13}C]JA for use as an internal standard. By using this compound, levels of JA have been quantified in tissues of developing soybean seedlings and in flowering plants. Furthermore, loss of cell turgor pressure during water deficit was found to stimulate the transient accumulation of JA. These data and others (3, 43) suggest that JA is derived from more than one source within cells to fulfill its multiple roles in plant development and defense.

MATERIALS AND METHODS

Plant Material. Soybean [*Glycine max* (L.) Merr. cv. Williams] seedlings were grown in darkness at 28°C and 100% relative humidity as described (8, 16). Tissue sections were excised under a green safelight and immediately frozen in liquid N_2 and stored at -80°C until extracted. Soybean plants were grown as described (8). Leaf number is as defined by Mason and Mullet (16) with leaf number 1 the most mature and leaf 7 the youngest. Flowers were harvested after petals opened. Soybean fruits were harvested 12 days after pollination [corresponding approximately to stage III (33)] and were separated into pericarp and seed. Tissue was immediately frozen in liquid N_2 and stored at -80°C until extracted. Fully mature dry seeds were removed from storage at 4°C and extracted without any further preparation.

For water deficit experiments, mature fully expanded leaves (leaf numbers 1 and 2) were excised from plants and were dehydrated by reducing their fresh weight by 15% with an air stream (\approx 15 min). Leaves were turned frequently so that both sides were exposed to the air stream to ensure equal dehydration. Control and stressed leaves were stored in plastic bags for various time periods. Some control leaves were turned over frequently without dehydration. Other leaves were stressed and immediately rehydrated. Tissue was immediately frozen in liquid N_2 and stored at -80°C until extracted. All experiments were performed at least twice with similar results.

Transpirational water loss from soybean leaves over a 4-h period was determined volumetrically. Similarly sized mature leaves were placed in graduated test tubes containing water, 100 μM abscisic acid (ABA), or 1 μM , 10 μM , 100 μM , or 500 μM JAMe. Water loss was determined periodically. The volume of water loss from the solutions in these tubes was subtracted from tubes lacking leaves to determine the loss due to transpiration in each leaf.

Extraction and Quantitation. For JA analysis, tissue was homogenized in acetone and filtered, and 374 ng of 2-(\pm)-[^{13}C]JA was added. After addition of 40–50 ml of distilled water, the extracts were rotary evaporated to the aqueous phase. Samples were brought to 100 mM potassium phosphate (pH 7.5) and the pH of the extract was then lowered to 2.5 with 6 M HCl. A suspension of DEAE-cellulose [100 mM potassium phosphate (pH 2.5)] was added and the extract was filtered. This step permitted removal of the bulk of the chlorophyll in

the samples. Samples were then partitioned three times against equal volumes of chloroform. The chloroform phase was dried over anhydrous sodium sulfate and removed by rotary evaporation. The crude extract was dissolved in a small volume of 90% hexane/10% ethyl acetate/1% acetic acid and further purified by using a Whatman amino column (4.6×216 mm) with a flow rate of 1 ml/min. The solvent composition was held at initial conditions (90% hexane/10% ethyl acetate/1% acetic acid) for 5 min followed by a linear gradient to 30% hexane/70% ethyl acetate/1% acetic acid in 10 min. Solvent composition was held at these final conditions for 15 min. Fractions corresponding to JA (26–28 min) were collected, dried, and methylated by using ethereal diazomethane. After methylation, samples were further purified by using a Waters silica gel column (3.9×150 mm) with a flow rate of 1.5 ml/min. Initial solvent composition was 100% hexane with a gradient to 90% hexane/10% ethyl acetate in 5 min. Solvent composition was held at these final conditions for 10 min. The fraction corresponding to JAme (12–13 min) was collected, dried, dissolved in ≈ 20 ml of dichloromethane, and analyzed by GC/MS/selected ion monitoring (20), monitoring m/z 83, 95, 151, 224, and 225 with a dwell time of 100 msec for each ion. Corrections were made for the natural abundance of stable isotopes by using a calibration curve with various amounts of unlabeled and ^{13}C JA (34) or by using a modification of the isotope dilution equation. Since 7-iso-JA can isomerize to JA during extraction, peak areas corresponding to these two isomers were combined to give a total amount of both isomers. Generally, JA and 7-iso-JA were present at a 9:1 ratio.

For ABA determinations, leaf tissue was lyophilized and extracted as described (20). After removal of the extraction solvent, the crude aqueous material was frozen and lyophilized. The residue was dissolved in a small volume of 90% distilled water/1% acetic acid/10% methanol and purified on a Waters C_{18} semiprep column (7.8×300 mm) at a flow rate of 2.5 ml/min. Initial conditions were 90% distilled water/1% acetic acid/10% methanol with a gradient to 20% distilled water/1% acetic acid/80% methanol in 25 min. The solvent composition was held at the final conditions for 5 min. Fractions corresponding to ABA were collected (24–26 min), dried, methylated with ethereal diazomethane, and analyzed by GC/electron capture detection as described (20). The recovery of ABA ranged from 50 to 70%.

Synthesis of (\pm) - ^{13}C JA. All chemicals were obtained from Aldrich unless otherwise noted. 1-Bromohex-3-ene was synthesized from phosphorus tribromide and hex-3-ene-1-ol (Bedoukian Research, Danbury, CT). Synthesis of 2-(pent-2-

enyl)cyclopent-2-en-1-one was performed as described by Dubs and Stüssi (35). Freshly distilled acrolein was added to a Grignard reagent prepared from Mg and 1-bromohex-3-ene to give crude nona-1,6-dien-3-ol. This compound was oxidized by using Jones reagent to give crude nona-1,6-dien-3-one. Sodium methoxide was added to a mixture of nona-1,6-dien-3-one and nitromethane in methanol to give 1-nitrodec-7-en-4-one, which was then subjected to a Nef reaction to give 4-oxodec-7-en-1-al. Base-induced intramolecular aldolization/dehydration of this keto aldehyde gave, in 2.2% yield, 180 mg of the desired cyclopentane product, which was purified by using silica gel chromatography. Pure 2-(pent-2-enyl)cyclopent-2-en-1-one was used in a Michael addition with 2- ^{13}C diethyl malonate (99.5 atom % ^{13}C), which was then subjected to decarboxylative saponification to give 2-(\pm)- ^{13}C JA as described by Knöfel and Gross (36). Mass spectra of intermediates and the final product were identical to published spectra (Fig. 2) (35).

RESULTS AND DISCUSSION

Synthesis of 2-(\pm)- ^{13}C JA. A high degree of enrichment and stable incorporation was obtained with 2-(\pm)- ^{13}C JA produced from 2-(pent-2-enyl)cyclopent-2-en-1-one and 2- ^{13}C diethyl malonate with an isotopic composition of [225]:[224] 0.98:0.02 compared with [225]:[224] 0.15:0.85 for natural material (Fig. 2). The enrichment value was lower (1.5%) than that predicted, suggesting that the actual enrichment of 2- ^{13}C diethyl malonate was less than that indicated by the supplier. Isomeric composition (determined by GC/flame ionization detection and MS) was judged to be 92% 2-(\pm)- ^{13}C JA and 8% 2-(\pm)-7-iso- ^{13}C JA. It would have been preferred to synthesize an internal standard containing several heavy isotopes, since ions from these will be distinct from those occurring in endogenous compounds through natural isotopic abundances. However, (\pm)-JA containing more than one atom of ^{13}C could not be synthesized due to a lack of commercially available intermediates containing heavy isotopes. It should be possible to introduce two deuterium atoms during the Michael addition and subsequent decarboxylative saponification if deuterium-enriched solvents and 2- $^{2}\text{H}_2$, ^{13}C diethyl malonate were used. However, chromatographic fractionation may occur when deuterium is used. No fractionation was observed with 2-(\pm)- ^{13}C JA. The synthesis 2- ^{14}C JA has been described (34); however, the reported specific activity (0.4 GBq/mmol) is too low to permit its use as an internal standard. The

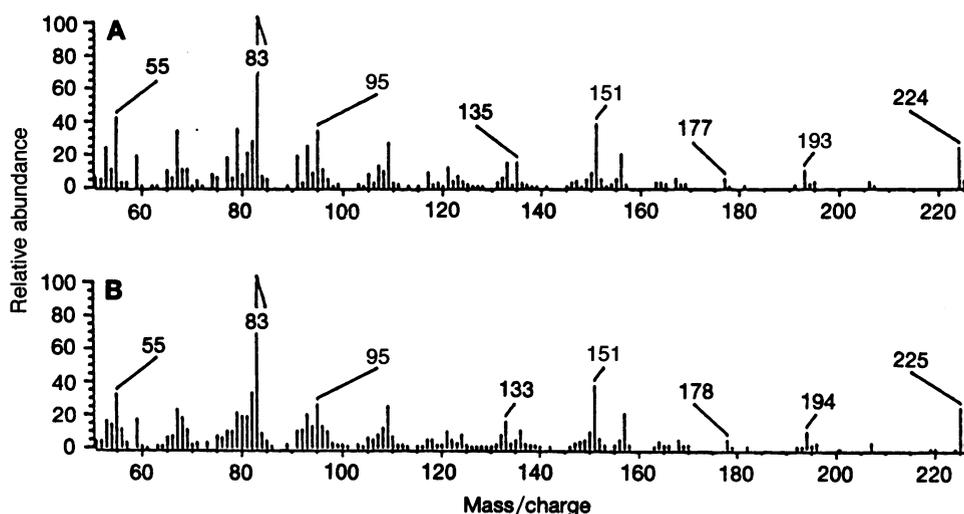


FIG. 2. Mass spectra of authentic JA (A) and 2-(\pm)- ^{13}C JA (B). Both samples were methylated prior to GC/MS analysis.

use of 2-(±)-[¹³C]JA as an internal standard for (-)-JA analysis by GC/MS/selected ion monitoring should be a significant improvement in the application of MS techniques for precise measurement of endogenous JA levels in plant tissues. This method is invaluable for studies of hormonal responses in plants and provides a reference for the evaluation of simpler, faster, or more sensitive techniques such as chromatographic or immunological methods. We have used it to quantitate JA levels in various tissues of soybean plants and in partially dehydrated leaves.

JA Levels in Vegetative Tissues. In soybean seeds and seedlings, JA levels were highest in the youngest tissues (hook, plumule, or 12-h axis; Fig. 3). Lower levels of JA were found in roots although root tips contained significant levels of the hormone. The distribution of JA in these same tissues is similar (8). In more mature soybean plants, JA levels were highest in young leaves, flowers, and fruit (Fig. 4). Levels of JA were the highest in the pericarp. The level of JA in this tissue determined by these methods [980 ng/g (fresh weight)] is close to that determined by radioimmunoassay [1180 ng/g (fresh weight)]; calculated from data in ref. 35].

In general, VSPA/B mRNA levels were elevated in tissues that contain high levels of jasmonate (ref. 17; for review, see ref. 19). Evidence that jasmonate limits *VspA/B* expression was previously obtained by demonstrating that addition of JA to tissues causes an induction of *Vsp* expression (16). However, in some tissues, JA level was not correlated with VSPA/B mRNA abundance. Little or no VSPA/B mRNA was detected in dry seeds, 12-h axis, mature root, and root tip (16), yet these organs contain relatively high JA levels. This indicates that in these organs additional factors or other plant growth substances regulate *VspA/B* expression. Auxin and phosphate have been demonstrated to inhibit JA-induced accumulation of VSP mRNA (22, 23).

JA Induction and the Response to Water Deficit. Reinbothe *et al.* (18) noted that several JA-induced peptides shared homology with late embryogenesis abundant proteins and could also be induced by water stress or ABA. In soybean leaves that had been stressed by allowing them to lose 15% of their fresh weight, JA levels increased ≈5-fold within 2 h and declined to approximately control levels by 4 h (Fig. 4). Levels of JA in dehydrated leaves were low 24 h after imposition of stress (data not shown). In contrast, a lag time of 1–2 h occurred before ABA accumulation reached a maximum. ABA levels remained high up to 8 h (Fig. 5). In addition, exogenous JA did not affect endogenous ABA levels (data not shown). The possibility that JA accumulation was induced by "wounding" the

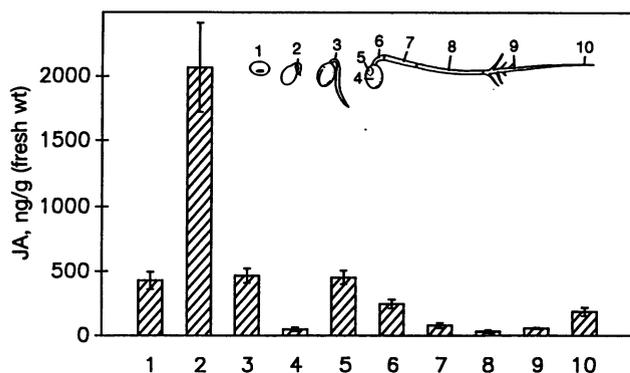


FIG. 3. JA levels in soybean seeds, germinating seeds, and seedlings. Soybean seedling stage or organ is defined as follows: Bars: 1, dry seed; 2, 12-h germinated seedling axis; 3, 24-h germinated stem hook; 4, cotyledon; 5, plumule; 6, stem hook; 7, stem elongating region; 8, stem nongrowing region; 9, root nongrowing region; 10, terminal 15-mm root tip from 2-day-old etiolated soybean seedlings. Data are the mean ± SD.

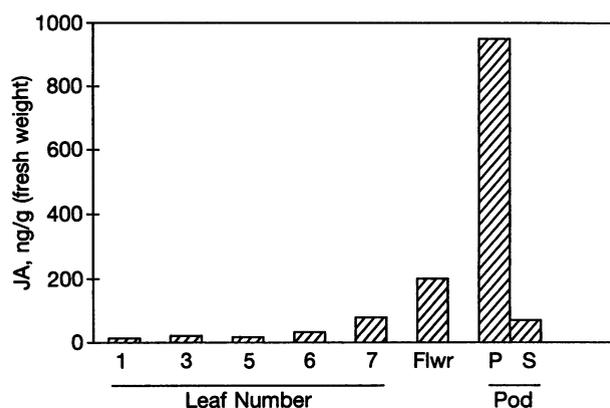


FIG. 4. JA levels in soybean leaves, flowers (flwr), and fruit (p, pericarp; s, seed). Data are the mean ± SD.

leaves as they were dehydrated does not seem likely. To determine whether any changes in JA resulted from the procedure used to stress leaves rather than the dehydration *per se*, turgid leaves were turned over with the same frequency as leaves being stressed or were dehydrated by 15% of their fresh weight and immediately rehydrated. No change in JA content occurred with these two treatments (data not shown), indicating that the increase in JA observed during dehydration does not result from wounding (20) or mechanical stimulation (13).

To determine whether JA was active as a promoter of stomatal closure (1), JA solutions were presented to the transpiration stream of soybean leaves for 4 h. Whereas 100 μM ABA was effective in reducing the rate of transpiration 72% (from a control value of 5.0 ml/h to 1.4 ml/h), JA concentrations ranging from 1 to 100 μM had little effect (98–95% of the control rate). A concentration of 500 μM JA reduced the rate of transpiration 22%. The effect of high JA concentrations on transpiration probably results from a toxic effect since the decline in transpiration caused by 1 mM JA was not reversed by moving treated leaves to water (37).

The rapid induction of JA observed in water-deficient leaves may result from turgor loss and related changes in ion transport. Changes in ion flow are associated with changes in turgor (38, 39). Changes in ion transport and JA levels are also reported to occur with tendril curling (13, 40, 41). While this rapid increase in JA level may mediate transient responses to reduced turgor, jasmonates are not involved in stomatal closure in soybean or barley (37). Because JA levels return to nonstressed levels within 4 h, it is likely that JA does not modulate longer-term adjustments to water deficit in this tissue. Changes in VSPA/B mRNAs are observed in the elongating regions of soybean seedlings 24 h after transfer to

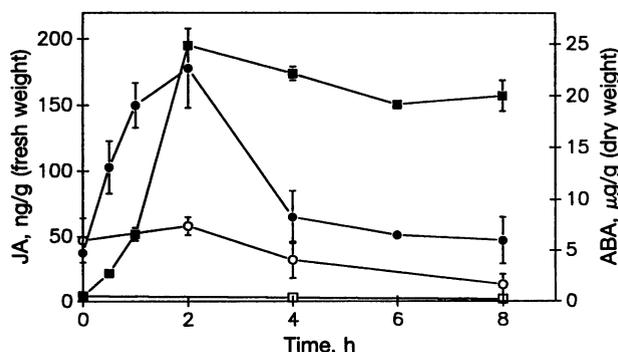


FIG. 5. JA (circles) and ABA (squares) levels in control turgid leaves (open symbols) or dehydrated leaves (solid symbols). Data are the mean ± SD.

low-water-potential vermiculite. Little change in JA levels in this tissue at 24 h was detected (data not shown). This result suggests accumulation of soluble sugars in this tissue in response to water deficit is sufficient to account for the increase in VSPA/B mRNA (21).

Multiple Sources and Functions for JA. JA levels in plant tissues vary with development, tissue type, and presence or absence of external stimuli (wounding, pathogens, or mechanical) (for review, see refs. 1–4). In addition, the genes regulated by JA range from those that encode proteinase inhibitors, fungal inhibiting proteins, and enzymes in phytoalexin biosynthesis to vegetative storage proteins and the large subunit of ribulose biphosphate carboxylase. The variety of responses and genes regulated by JA suggests the existence of multiple levels of control over jasmonate biosynthesis and that JA acts with other effectors to potentiate gene expression. In the following section, we discuss evidence that JA originates from plasma membranes and plastid membranes and attempt to rationalize why jasmonate regulates the expression of genes involved in pathogen or insect defense as well as vegetative protein storage and photosynthesis.

Ryan (24) has proposed that JA biosynthesis is stimulated by pathogens or insect pests through the production of elicitors and systemic signaling molecules that interact with specific receptors on the plasma membrane (Fig. 6). This model is consistent with the finding that carbohydrate elicitors induce accumulation of jasmonate in cell culture (25). In addition, the peptide elicitor systemin induces the jasmonate-responsive *pinII* gene at very low concentrations (42). Systemin-mediated induction of *pinII* is blocked by inhibitors of JA biosynthesis (24). The model further proposes release of linolenic acid from plasma membrane lipids by lipase action with subsequent conversion of fatty acids to JA by the action of cytosolic lipoxygenase, allene oxide synthase, allene oxide cyclase, and 12-oxo-PDA reductase (Fig. 6). The final steps in JA biosynthesis involving β -oxidation presumably occur in microbodies. One could propose a similar pathway for the synthesis of JA in wounded tissues where disruption of the cell wall releases oligosaccharide inducers. Alternatively, wounding may simply mix hydrolytic enzymes, lipids, or fatty acids located in vacuoles, plastids, and the cytoplasm, which results in JA biosynthesis. JA induced by mechanical stimulation (touching or tendrils coiling) or loss of turgor could also be derived from the

plasma membrane. In these cases, one could postulate that mechanical stimulation and loss of turgor stimulates lipase or lipoxygenase activity in plasma membranes through alteration of ion channels sensitive to membrane perturbation. Interestingly, mechanical stimulation of tendrils resulted in a decrease in monogalactosyldiacylglycerol and phosphatidylinositol, potential precursors of JA (13). Monogalactosyldiacyldiglyceride is nearly exclusively located in plastids. Therefore, if this lipid is a source of linolenic acid for jasmonate biosynthesis, then plastid membranes must be considered the most likely starting point for synthesis. Other information is consistent with plastids playing a role in JA biosynthesis. (i) Lipoxygenase is located in plastids, vacuoles, and the cytoplasm (3, 43). (ii) Early studies by Vick and Zimmerman (3) revealed that plastids contain activities corresponding to allene oxide synthase and allene oxide cyclase. This suggests that there are two pathways for the initial steps in JA biosynthesis, one starting from the plasma membrane and the other starting from plastid membranes (Fig. 6).

The induction of many jasmonate-responsive genes (i.e., *Lox*, *Vsp*, or *pinII*) by jasmonate is stimulated by sugars (sucrose, glucose, or fructose) and by illumination of plants (23, 44). The influence of light on *Vsp* expression can be partially blocked by DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea], an inhibitor of photosynthetic electron transport (16). These results suggest a connection between photosynthesis and one role of JA in plants. Photosynthesis is inhibited if sucrose export from cells is inhibited or if sugars are taken up by cells (for review, see ref. 45). In this situation, sugar phosphate levels in the cytoplasm increase and cytoplasmic phosphate levels decrease, limiting release of triose phosphate from plastids via the phosphate translocator. This stimulates starch biosynthesis and eventually results in a decrease in carbon fixation in chloroplasts. Under these conditions, the products of photosynthetic electron transport are no longer utilized for carbon fixation and the energy harvested by the chlorophyll antennae must be dissipated rather than used for the formation of ATP and reducing power. Some of the excess energy can be dissipated via the xanthophyll cycle or through other energy quenching reactions (46). Even so, superoxide and hydroxyl radicals are generated under these conditions at high light. Superoxide dismutase, ascorbic acid, and glutathione generating systems are present in plastids to minimize the damage caused by superoxide and oxygen radicals. However, once the capacity of these systems is exceeded, membrane damage will occur. Lipoxygenase and other enzymes that metabolize fatty acids may protect membranes from further damage by removing these products. The lipoxygenase-mediated generation of JA could, in turn, induce changes in the cell that ameliorate further photochemical damage. For example, jasmonate induced loss of chlorophyll (1) would decrease the amount of light energy absorbed by the photosynthetic apparatus. The accumulation of anthocyanins that is stimulated by JA in illuminated plants (47) could provide some protection from excess radiation. Moreover, induction of vegetative storage protein synthesis under conditions of high sugar and low phosphate would create a sink for carbon and nitrogen releasing a phosphate from the sugar phosphate pools for further carbon fixation. Interestingly, two of the vegetative storage proteins are lipoxygenase and acid phosphatase, enzymes that could mobilize phosphate from lipids and phosphate esters, respectively. Furthermore, jasmonate-mediated inhibition of *rbcL* expression and other genes encoding proteins involved in photosynthesis is consistent with the diminished need for photosynthate in high light and excess carbon. Finally, water deficit induces closure of stomata, limits internal CO₂ levels, and inhibits carbon fixation. This situation can also result in an imbalance in the absorption and utilization of light energy. Therefore, the activation of jasmonate-responsive genes under conditions of water deficit may not be surprising (for review, see ref. 1).

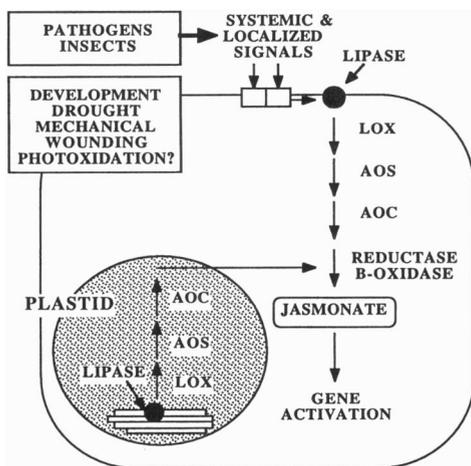


FIG. 6. Diagram showing two potential pathways for synthesis of JA. Pathogens and insects are shown to produce localized and systemic elicitors that interact with plasma membrane receptors as described by Ryan (24). Receptor stimulation triggers lipase-mediated release of linolenic acid followed by conversion to JA by the concerted action of lipoxygenase (LOX), allene oxide synthase (AOS), allene oxide cyclase (AOC), 12-oxo-PDA reductase, and β -oxidation. A similar pathway is proposed to originate from plastid membranes.

Many of the JA-responsive genes are highly expressed in hypocotyl hooks, flowers, and young developing fruits (19). These tissues are known to be very active sinks for carbon and nitrogen and to contain high levels of JA. In these tissues, JA biosynthesis and accumulation must be under developmental control. The accumulation of jasmonate-induced proteins such as the proteinase inhibitors in developing fruit could offer some level of constitutive defense against insects. Other expression patterns for JA-responsive genes are apparently caused by specific combinations of responsive elements. For example, osmotin is induced to very high levels by ethylene and JA but each compound alone is much less effective (28). This suggests that responsiveness to JA has been combined with other effector pathways to further specialize the conditions under which genes respond to this plant hormone.

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