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Jasmonic Acid-Dependent Increase in Vegetative Storage Protein in Soybean Tissue Cultures

James Michael Anderson

United States Department of Agriculture, Agricultural Research Service, Departments of Crop Science and Botany, North Carolina State University, Raleigh, North Carolina 27695-7631, USA

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Abstract. Adding jasmonic acid (JA) to autotrophic, photomixotrophic, or heterotrophic suspension cultures of soybean specifically increased the level of the M_r 30,000 subunit of soybean vegetative storage protein (VSP-30) and a polypeptide at M_r 18,000 that interacted with antibody raised against VSP. Using photomixotrophic cells, the increase was observed at concentrations as low as 10 nM JA and the increase was evident within 2 h following treatment. Below 10 μM, JA did not inhibit growth of the cells but did cause browning at higher concentrations. Other plant growth regulators, including abscisic acid (ABA), gibberellic acid, and benzyl adenine, did not alter the level of VSP-30 either in the presence or absence of JA. Methyl jasmonate (JA-Me), 3-oxo-2-butyl-cyclopentane-1-acetate, and 3-oxo-2-pentyl-cyclopentane-1-acetate also increased VSP-30 but at higher concentrations than JA. Altering the level of reduced nitrogen or sucrose in the medium did not alter VSP-30 levels in the cells, but at higher sucrose concentrations, sensitivity to JA was reduced. The dramatic increase in VSP-30 elicited by JA appears to be a specific response to the phytohormone.

There is increasing evidence that jasmonic acid (JA) is a natural plant growth regulator in higher plants (Anderson 1989, Vick and Zimmerman 1987). Early experiments showed that JA, or its methyl ester (JA-Me), inhibited the growth of several tissue explants (Ueda and Kato 1982, Yamane et al. 1981) and could also increase the rate of senescence of some isolated tissues (Ueda et al. 1981, Weidhase et al. 1987b). Later work has shown that JA induces the synthesis of specific polypeptides in both isolated and intact plant tissues from grasses (Weidhase et al. 1987a), composites (Anderson 1987), and legumes (Anderson 1988). The polypeptides induced in barley leaf segments appear to be similar to, but distinct from, heat-shock proteins (Mueller-Uri et al. 1988). Although no function has been identified for any of these JA-increased polypeptides, the similarity between JA-increased proteins and heat-shock proteins suggests that JA, or its methyl ester, may be involved as a signal in some stress-related process. The transduction of the JA-stress signal could ultimately lead to senescence.

In intact soybean leaves, JA specifically increases the level of two polypeptides that have been identified as subunits of a vegetative storage protein (VSP; Anderson et al. 1989). Soybean VSP normally is found in the leaf paraveinal mesophyll (Franceschi et al. 1983) and appears to function as a temporary nitrogen store that accumulates during vegetative growth and disappears during pod fill (Staswick 1989). Although JA has been observed to cause small increases in the level of several other polypeptides in soybean leaves (Anderson 1988), all of these polypeptides appear similar to proteins that increase with depodding and therefore must be similar to VSPs (Wittenbach 1983) and not stress-related proteins.

Soybean VSP consists of a random mixture of two dissimilar glycopeptides (Spilatro and Anderson 1989), and JA increases the level of one of these glycopeptides in photomixotrophic suspension cul-

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tures of soybean (Anderson 1989). In contrast to barley explants (Weidhase et al. 1987a), JA increases the level of VSP in cultured soybean cells at concentrations much lower than those required to affect growth or induce the senescence of the cells (Anderson 1989). The cultured cells provide a unique system for studying the phytohormonal properties of JA because both the level of JA and the tissue response to it can be defined to a high degree. This report characterizes the JA-dependent increase of VSP in cultured soybean cells and attempts to determine if the increase in VSP is caused directly by JA or by some secondary process, such as alteration in the level of another plant growth regulator.

Materials and Methods

Chemicals

Methyl jasmonate, (±)-JA-Me, was the kind gift of Dr. Gunther Ohloff of Firmenich SA, Geneva, Switzerland. (±)-Jasmonic acid, JA (3-oxo-2-(2'-*cis*-pentenyl)-cyclopentane-1-acetate), was made by alkaline hydrolysis of (±)-JA-Me (Anderson 1985). The jasmonic acid isomers, 3-oxo-4-pentyl-cyclopentane-1-acetate, 3-oxo-2-butyl-cyclopentane-1-acetate, and 3-oxo-2-propyl-cyclopentane-1-acetate (Yamane et al. 1980), were a kind gift from Dr. N. Takahashi, University of Tokyo, Japan. All JA isomers except JA-Me were dissolved in water as the potassium salts and filter sterilized. JA-Me was dissolved in ethanol and filter sterilized prior to inclusion in culture media. Appropriate ethanol controls were tested in each JA-Me experiment to rule out toxicity effects of the solvent.

Tissue Culture

Suspension cultures of soybean (*Glycine max* var. Corsoy) were originally supplied by Dr. Jack Widholm, University of Illinois (Horn et al. 1983). When cells were grown photomixotrophically or heterotrophically, a cell line that had lost the ability to grow autotrophically due to years of mixotrophic growth was used (Spilatro and Anderson 1988). The autotrophic cell line was a reisolate from the photomixotrophic cell line. A cell line devoid of chlorophyll was selected from cells grown heterotrophically for several generations in the dark.

Cultures were grown in media consisting of Murashige and Skoog salts and vitamins (Sigma Chemical Co., St. Louis, MO, USA) containing 0.35 μM benzyladenine and 5.0 μM napthalene acetic acid (NAA) (Spilatro and Anderson 1988). Cells were grown autotrophically with no added sucrose, photomixotrophically with 0.4% wt/vol sucrose, and heterotrophically with 3% sucrose. When glutamine was used as the sole nitrogen source, it was added at 30 mM to medium made without NH_4NO_3 or KNO_3. K_2SO_4 was added at 10 mM to give the same potassium ion concentration as in the unmodified medium. The glutamine medium was filter sterilized. Cell density of each culture was measured by allowing cultures to settle for 20 min in 100-ml graduates. Experiments were initiated by adding 8 ml of settled cells plus 42 ml of old medium to 50 ml of fresh medium. Filter-sterilized test compounds were added at 24 h after subculture, and experiments were terminated 48 h after adding test compounds by collecting cells on nylon nets, rinsing with H_2O, and blotting dry. Tissue was frozen in liquid N_2 and lyophilized.

Protein Extraction

Lyophilized plant material (0.25 g) was repeatedly extracted with ice-cold acetone using a ground-glass homogenizer until free of visible chlorophyll. The resulting powder was dried on filter paper, and homogenized in 5 ml of ice-cold buffer containing 50 mM Tris-HCl (pH 7.6), 3 mM EDTA, 2% wt/vol insoluble polyvinylpyrrolidone, and 700 μM β-mercaptoethanol. Homogenates were filtered though four layers of cheesecloth and any remaining precipitate was removed by centrifugation at 18,000 *g* for 20 min. A 1-ml aliquot of each sample was desalted through G-25 Sephadex using centrifugation.

Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was done using the methods of Laemmli (Laemmli 1970). Samples of 60 μg, as determined by the method of Bradford (Bradford 1976), were separated in 13% acrylamide by electrophoresis. Protein bands were resolved with Coomassie blue R-250 (Bio-Rad, Richmond, CA, USA). Protein was electroblotted from a duplicate gel to a nitrocellulose membrane as previously described (Spilatro and Anderson 1988). Membranes were probed with rabbit anti-VSP that had been affinity purified (Spilatro and Anderson 1989). Immunoreactive bands were visualized using horseradish peroxidase-conjugated secondary antibody. Wet Western blots were scanned using a laser densitometer (LKB-Ultrascan XL) and relative amounts of VSP in each blot were related to three separate concentrations of a VSP standard in the same blot. Blots were developed to a density that gave approximately a linear relationship between the concentrations of the three standards, and the data for experimental samples were used only if the densities were in the same range as for the standards.

Results

Jasmonic Acid-Dependent Increase in VSP

Previous results showed that adding JA to soybean photomixotrophic suspension cultures increased the level of three polypeptides at M_r 18,000, 30,000, and 88,000 (Anderson 1988, Anderson et al. 1989). The 18 and 30 kD polypeptides (VSP-18 and VSP-30) interacted with affinity purified VSP antibodies and both polypeptides clearly were increased by addition of JA to the culture medium (Fig. 1). The 30 kD polypeptide has been identified as the large subunit of soybean VSP (Anderson et al. 1989), but a VSP subunit has not been identified at 18 kD. VSP-18 is rarely seen in JA-treated leaf tissue and, therefore, this polypeptide may represent a degradation product of VSP-30 that is unique to cultured cells. Because of the uncertainty about VSP-18, the data

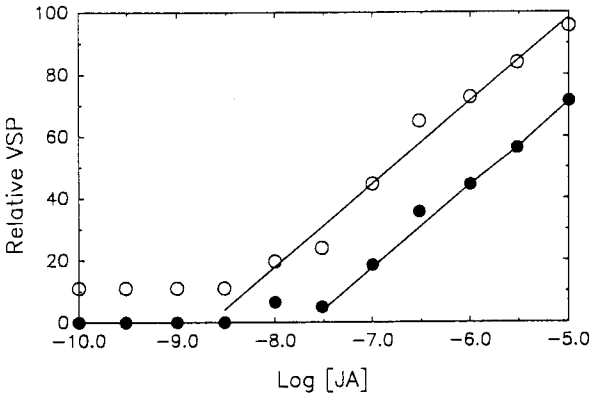

Fig. 1. Increase in the level of VSP elicited by JA treatment of soybean photomixotrophic suspension cells. Soluble protein (65 μg/well) extracted from the cells was separated by SDS-PAGE, blotted to nitrocellulose, probed with affinity-purified anti-VSP, and quantitated by scanning with a densitometer. VSP bands were quantified at M_r 30,000 (○——○) and M_r 18,000 (●——●). Three VSP standards were run on the gel to standardize the densitometric data. Data shown are the average of two separate experiments.

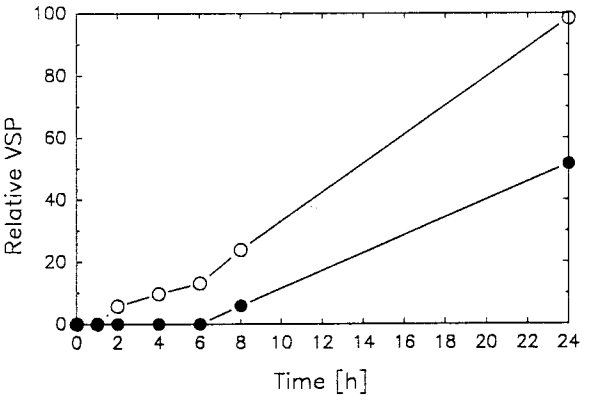

Fig. 2. Time course of the increase in VSP levels elicited by JA treatment of soybean photomixotrophic suspension cells. Soluble protein (65 μg/well) extracted from the cells was separated by SDS-PAGE, blotted to nitrocellulose, probed with affinity-purified anti-VSP, and quantitated by scanning with a densitometer. VSP bands were quantified at M_r 30,000 (○——○) and M_r 18,000 (●——●). Three VSP standards were run on the gel to standardize the densitometric data.

for Figs. 1 and 2 were calculated as relative VSP in the extract rather than μg VSP. Although the VSP subunit at M_r 29,000 does interact with the antibody (Spilatro and Anderson 1989), under no circumstances was this polypeptide ever observed to increase in cultured cells with any treatment. The 88 kD polypeptide does not interact with the antibody and, therefore, was not measured.

JA at levels as low as 10 nM produced a significant increase in VSP-30, and the rate of increase was linear with the log of the JA concentration to at least 10 μM JA (Fig. 1). The relative increase in VSP-18 was also linear, but it occurred at JA concentrations an order of magnitude higher than those required to increase VSP-30. While some of the cultures had low basal levels of VSP without JA treatment (Fig. 1), this usually was not the case, and the concentration curve shown in Fig. 1 has been reproduced in over four separate experiments. At levels greater than 10–30 μM, JA was toxic to the cultures, inhibited growth, and caused browning of cells (data not shown).

When cells were treated with 10 μM JA, an increase in the level of VSP-30 could be visualized after 2 h (Fig. 2). After an 8 h treatment, an increase in VSP-18 was also detectable. The level of both VSP-30 and VSP-18 continued to increase in a linear fashion for about 24 h after adding JA.

Culture Conditions and VSP Levels

Treatments that hinder the export of nitrogen from soybean leaves, such as removal of sinks by depodding, cause VSP to accumulate in the leaves (Staswick 1989). These results might indicate that VSP levels are regulated in vivo by the level of free amino acids in the leaf. However, in cultured cells, VSP accumulation was not elicited by altering the level of amino acids in the culture medium by adding casein hydrolysate (Table 1). Even if all of the nitrogen in the medium was added as glutamine, VSP accumulation was not increased (Fig. 3). In fact, the cultured cells grown in glutamine had about the same sensitivity to JA as cells grown in inorganic nitrogen.

The concentration of sucrose in the culture medium does not alter the rate of growth of the cells but does determine how long the cells continue to divide (Spilatro and Anderson 1988). Most of the experiments that tested the sensitivity to JA treatment were done using sucrose concentrations ranging between 6 and 10 mM. This concentration range supports log-phase growth for about 3–4 days, which corresponds to the length of an experiment (data not shown). Increasing the level of sucrose in the medium caused the cells to grow heterotrophically (Spilatro and Anderson 1988). Cells grown heterotrophically did not have an altered level of VSP in the absence of JA, but the cells did show a lowered sensitivity to JA (Fig. 3). At higher sucrose concentrations, the slope of VSP versus log[JA] was increased, and the toxic effects of JA on growth of the cells occurred at a lower concentration (Fig. 3).

JA also increased the level of VSP-30 in cells grown autotrophically without added sucrose

Table 1. Changes in VSP levels elicited by additions to the culture medium

Additive	Concentration (—μM) −JA Range	−JA Effective	+JA Range	+JA Effective
Control	—	—	0.01–50	0.01
ABA	1–30	None	1–30	1
Benzyl adenine	1–30	None	1–50	1
Gibberellic acid-3	1–30	None	—	—
Amino acids (as casein hydrolysate)	0–6,300	None	7	7

Cells were grown in media 24 h prior to adding the test compound. All test compounds were added at decreasing increments of 0.5* log (concentration) over the range shown except for the nutrients which were tested at concentrations shown. The effect of the additive on the JA-dependent increase in VSP was also tested using the range of JA concentrations shown. The effective concentration was determined as that concentration that increased the level of VSP to 0.15 μg VSP/mg soluble protein.

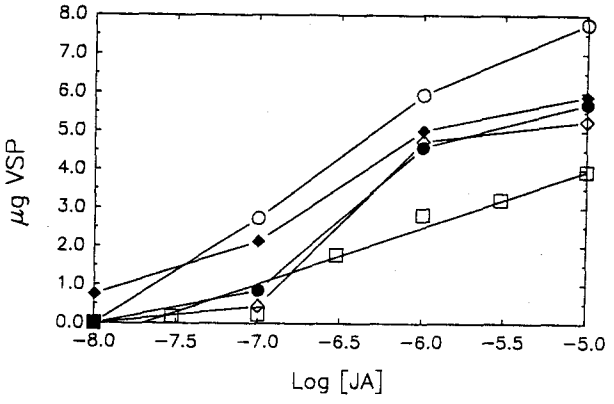

Fig. 3. Increase in VSP levels elicited by JA treatment of soybean photomixotrophic suspension cells grown with different nutrient conditions. Soluble protein (60 μg/well) extracted from the cells was separated by SDS-PAGE, blotted to nitrocellulose, probed with affinity-purified anti-VSP, and quantitated by scanning with a densitometer. Three VSP standards were run on each gel to standardize the densitometric data and calculate the μg VSP/mg soluble protein. Cells were grown in no sucrose (□——□), 5.8 mM sucrose (○——○), 29 mM sucrose (●——●), or 58 mM sucrose (◇——◇) and treated for 48 h with JA. Also, cells were grown in 6 mM sucrose with 50 mM glutamine (♦——♦) added as the sole nitrogen source.

(Fig. 3). However, the maximum level of VSP accumulated was lower than in photomixotrophic cells. Dark-grown cells which were devoid of chlorophyll showed a VSP increase with JA treatment that was similar to cells grown heterotrophically in the light (data not shown).

Effect of Other Growth Substances

Abscisic acid (ABA) has similarities in both chemical structure and biological activity to JA (Anderson 1989) and might be expected to mimic the effects of JA. Also, the level of JA proteins in barley leaf explants can be increased with ABA (Mueller-Uri et al. 1988). However, at concentrations ranging from 1–30 μM, ABA had no effect on the level of VSP either in the presence or absence of JA (Table 1). Also, benzyladenine and gibberellic acid-3 did not alter the level of VSP at concentrations ranging from 3–30 μM.

Table 2. Changes in VSP levels elicited by analogs of JA

Additive	Concentration Range (μM)	Lowest effective (μM)	Level of VSP at 3.0 μM additive (μg)
Control	—	—	0
JA (3-oxo-2-(2′-*cis*-pentenyl)-cyclopenate-1-acetate)	0.01–50	0.01	0.95
JA-Me (3-oxo-2-(2′-*cis*-pentenyl)-cyclopentane-1-methylacetate)	0.01–50	1.0	0.30
Butyl-jasmonic acid (3-oxo-2-butyl-cyclopentane-1-acetate)	3–30	3	0.18
Propyl-jasmonic acid (3-oxo-2-propyl-cyclopentane-1-acetate)	3–30	9.3	0.05
Iso-jasmonic acid (3-oxo-4-pentyl-cyclopentane-1-acetate)	3–30	No effect	0

Cells were grown in media 24 h prior to adding the test compound. All test compounds were added at decreasing increments of 0.5* log (concentration) over the range shown. The effective concentration was determined as that concentration that increased the level of VSP to 0.15 μg VSP/mg soluble protein. The level of VSP at 3.0 μM additive is also shown in the last column.

Analogs to JA

JA-Me has been shown to alter the level of polypeptides in barley leaf segments in a manner similar to JA (Weidhase et al. 1987a). However, with cultured soybean cells it required 30 times as much JA-Me to obtain the same increase in VSP-30 as was elicited by JA (Table 2). Three chemical isomers of JA, with modifications to the pentenyl side chain at position 2 of the cyclopentane ring (Yamane et al. 1980), were tested for their ability to increase VSP in the cultured cells. Two of the isomers which had shortened, saturated, side chains (butyl-JA and propyl-JA) increased VSP-30 but required much higher concentrations than JA (Table 2). A third isomer which had a pentenyl side chain at position 4 of the cyclopentane ring instead of the pentenyl at position 2 was devoid of activity.

Discussion

JA treatment specifically increases the level of several polypeptides in intact soybean leaves and photomixotrophic suspension cultures (Anderson et al. 1988). Of these two systems, suspension cultures provide a unique system for studying the regulation by a plant growth substance of the accumulation of a specific polypeptide. One of the polypeptides increased by JA in the cultured cell has been identified as VSP (Anderson et al. 1989). The amount of VSP accumulated in response to JA treatment often exceeds 0.1% of the total soluble protein in the cells, and the response is linear with JA concentrations over several orders of magnitude. If the cells are grown using a defined set of environmental conditions, a concentration curve can be repeated, even with a year separating experiments.

In intact plants, the sensitivity to a particular growth substance is dictated by both developmental and environmental conditions (Blowers and Trewavas 1989). In the cultured soybean cells, sensitivity to JA also appears to be environmentally regulated. Increasing the sucrose concentration in the culture medium appears to lower sensitivity to JA. A simple explanation might be that cells grown at higher sucrose concentrations are dividing faster, and, therefore, less protein is partitioned into VSP at any JA concentration. However, cells grown at 6 and 58 mM sucrose appear to divide at the same rate (Spilatro and Anderson 1988). At 6 mM sucrose, cells stop dividing after about 3 days of culture, which corresponds to the normal length of an experiment, rather than 6 days for 58 mM sucrose. Therefore, the developmental shift from heterotrophic growth to autotrophic subsistence may have already begun at the lower sugar concentration, and this shift may be responsible for the increased sensitivity to JA treatment. Changes in nutrient concentration could also alter the rate of JA uptake into the cells. Increased tissue sensitivity to growth substances during metabolic resource limitation has been noted previously (Trewavas 1986).

The effect of JA on VSP accumulation could occur by a direct transduction of the JA signal into increased synthesis of VSP message, or the JA signal could cause a primary response that leads secondarily to VSP accumulation. The former proposition is supported by the observation that the VSP message is increased by JA in the same mixotrophic cell line (Staswick 1989, personal communication). Also, a secondary messenger for the JA signal has not been found. Other growth substances including ABA, benzyladenine, gibberellic acid, and NAA have no effect. In fact, VSP accumulation is fairly specific for unmodified JA, as analogs of JA which are altered in the pentenyl side chain are only partially effective. Another possibility is that JA increases ethylene production in the cells and that the ethylene, in turn, increases VSP. However, a role for ethylene is unlikely because the atmosphere within the culture flasks is continuously flushed out, and changing the flushing rate did not alter the sensitivity of the cells to JA treatment (unpublished observation).

The physiological function of JA in plants has remained obscure in spite of several investigations over the last few years. The vast majority of these investigations have shown that JA can cause an inhibition of growth and in some excised tissues can induce senescence (reviewed in Anderson 1989). The senescence-inducing properties of JA have been studied most intensively using excised barley leaf segments floated on water (Popova and Vaklinova 1988, Weidhase et al. 1987b). When treated with JA or JA-Me, the rate of senescence is increased and the synthesis of a series of specific polypeptides is induced (Weidhase et al. 1987a). These JA-induced proteins resemble the heat-shock proteins and may play a role in the senescence induction process.

However, in soybean cultures or intact leaves, JA does not alter growth or increase the rate of senescence except at concentrations in excess of 10 μM. The inhibition encountered at 10 μM JA is probably a simple toxicity effect, since most other growth substances also become toxic at similar concentrations (unpublished results). In fact, the level of VSP is increased without tissue deterioration in excised soybean leaves which take up JA through the cut end of the petiole (unpublished results). Clearly, barley leaf segments and soybean cells react differently to JA treatment.

The cultured soybean cells are an actively growing tissue with a source of nutrients to maintain metabolism and biosynthetic activity. The excised barley leaves are deprived of a nutrient source and, therefore, are a dead-end system waiting to be pushed into senescence. In fact, ABA will substitute for JA in both the senescence induction and stimulation of JA proteins (Mueller-Uri et al. 1988). However, both tissues could react to the JA signal in a similar manner if JA functioned as a stress signal. The excised barley leaves have no options except senescence with which to react to a stress signal, but soybean leaves, which the cultured cells partially resemble, could react to a stress signal by storing excess nitrogen as VSP. By accumulating VSP during the early stages of a stress the plant would have more stored nitrogen with which to cope with latter stages of the stress when nitrogen supplies would be limiting.

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References

Anderson JM (1985) Simultaneous determination of abscisic acid and jasmonic acid in plant extracts using high-performance liquid chromatography. J Chromatogr 330:347–355

Anderson JM (1987) Jasmonic acid-dependent increase in specific polypeptides and polyphenoloxidase in three species of composites. Plant Physiol 83S:135

Anderson JM (1988) Jasmonic acid-dependent increase in the level of specific polypeptides in soybean suspension cultures and seedlings. J Plant Growth Regul 7:203–211

Anderson JM (1989) Membrane-derived fatty acids as precursors of second messengers. In: Boss WF, Morre DJ (eds) Second messengers in plant growth and development. Alan R. Liss, New York, pp 181–212

Anderson JM, Spilatro SR, Klauer SF, Franceschi VR (1989) Jasmonic acid-dependent increase in the level of vegetative storage proteins in soybean. Plant Sci 62:45–52

Blowers DP, Trewavas AJ (1989) Second messengers: Their existence and relationship to protein kinases. In: Boss WF, Morre DJ (eds) Second messengers in plant growth and development. Alan R. Liss, New York, pp 1–28

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Franceschi VR, Wittenbach VA, Giaquinta RT (1983) Paraveinal mesophyll of soybean leaves in relation to assimilate transfer and compartmentation. III. Immunohistochemical localization of specific glycoproteins in the vacuole after depodding. Plant Physiol 72:586–589

Horn ME, Sherrard JH, Widholm JM (1983) Photoautotrophic growth of soybean cells in suspension culture. I. Establishment of photoautotrophic cultures. Plant Physiol 72: 426–429

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685

Mueller-Uri F, Parthier B, Nover L (1988) Jasmonate-induced alteration of gene expression in barley leaf segments analyzed by in-vivo and in-vitro protein synthesis. Planta 176:241–247

Popova LP, Vaklinova SG (1988) Effect of jasmonic acid on the synthesis of ribulose-1,5-bisphosphate carboxylase-oxygenase in barley leaves. J Plant Physiol 133:210–215

Spilatro SR, Anderson JM (1988) Carbohydrate metabolism and activity of pyrophosphate:fructose-6-phosphate phosphotransferase in photosynthetic soybean (*Glycine max*, Merr.) suspension cells. Plant Physiol 88:862–868

Spilatro SR, Anderson JM (1989) Characterization of a soybean leaf protein that is related to the seed lectin and is increased with pod removal. Plant Physiol 90:1387–1393

Staswick PE (1989) Preferential loss of an abundant storage protein from soybean pods during seed development. Plant Physiol 90:1252–1255

Trewavas AJ (1986) Resource allocation under poor growing conditions: A major role of growth substances in developmental plasticity. In: Jennings DH, Trewavas AJ (eds) Plasticity in plants. Company of Biologists Ltd, Cambridge, pp 31–76

Ueda J, Kato J, Yamane H, Takahashi N (1981) Inhibitory effect of methyl jasmonate and its related compounds on kinetin-induced retardation of oat leaf senescence. Physiol Plant 52:305–309

Ueda J, Kato J (1982) Inhibition of cytokinin-induced plant growth by jasmonic acid and its methyl ester. Physiol Plant 54:249–252

Vick BA, Zimmerman DC (1987) Oxidative systems for modification of fatty acids: The lipoxygenase pathway. In: Stumpf PK, Conn EK (eds) The biochemistry of plants. Lipids. Vol. 9. Academic, New York, pp 53–90

Weidhase RA, Kramell HM, Lehmann J, Liebisch H-W, Lerbs W, Parthier B (1987a) Methyl jasmonate-induced changes in the polypeptide pattern of senescing barley leaf segments. Plant Sci 51:177–186

Weidhase RA, Lehmann J, Kramell HM, Sembdner G, Parthier B (1987b) Degradation of ribulose-1,5-bisphosphate carboxylase and chlorophyll in senescing barley leaf segments triggered by jasmonic acid methylester, and counteraction by cytokinin, Physiol Plant 69:161–166

Wittenbach VA (1983) Purification and characterization of a soybean leaf storage glycoprotein. Plant Physiol 73:125–129

Yamane H, Sugawara J, Suzuki Y, Shimamura E, Takahashi N (1980) Synthesis of jasmonic acid related compounds and their structure–activity relationships on the growth of rice seedlings. Agric Biol Chem 44:2857–2864

Yamane H, Takagi H, Abe H, Yokota T, Takahashi N (1981) Identification of jasmonic acid in three species of higher plants and its biological activities. Plant Cell Physiol 22:689–697