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Developmental and Tissue-Specific Expression of JIP-23, a Jasmonate-Inducible Protein of Barley

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Developmental expression of a 23 kDa jasmonate-induced protein (JIP-23) of barley leaves (*Hordeum vulgare* cv. Salome) was studied by measuring the time-dependent accumulation of transcript and protein during germination. Tissue-specific expression of JIP-23 was analyzed immunocytochemically and by in situ hybridizations, respectively. During seed germination JIP-23 mRNA was found to accumulate transiently with a maximum at 32 h, whereas the protein was steadily detectable after the onset of expression. The occurrence of new isoforms of JIP-23 during germination in comparison to jasmonate-treated leaves suggests, that the JIP-23 gene family of barley is able to express different subsets of isoforms dependent on the developmental stage.

JIP-23 and its transcript were found mainly in the scutellum, the scutellar nodule and in lower parts of the primary leaf of 6 days old seedlings. All these tissues exhibited high levels of endogenous jasmonates. In situ hybridization revealed specific accumulation of JIP-23 mRNA in companion cells of the phloem in the nodule plate of the scutellum. In accordance with that, JIP-23 was detected immunocytochemically in phloem cells of the root as well as of the scutellar nodule and in parenchymatic cells of the scutellum. The cell type-specific occurrence of JIP-23 was restricted to cells, which are known to be highly stressed osmotically by active solute transport. This observation suggests, that the expression of this protein might be a response to osmotic stress during development.

Key-words: Endogenous jasmonate level — Germination — *Hordeum vulgare* L. — Immunofluorescence analysis — In situ hybridization — Scutellar nodule.

Among jasmonates, a class of cyclopentanone compounds, mainly (−)-jasmonic acid (JA), its methyl ester (JM) and its amino acid conjugates are regarded to be putative regulators of plant growth and development (Sembdner and Parthier 1993). (−)-JA occurs ubiquitously in higher plants (Yamane et al. 1981, Meyer et al. 1984). Similar to abscisic acid (ABA), JA was suggested to be a signal functioning upon environmental stress. To elucidate signalling via JA and its function in altering expression of distinct genes, JA-inducible genes have been identified. Among them there are genes which express the wound-induced proteinase inhibitors I and II of tomato and potato, implicating a role of JA in the plant defence related to herbivore attack (Peña-Cortés et al. 1989, Ryan 1992). In contrast, N-partitioning in soybean via vegetative storage proteins is triggered also by JA (for review see Staswick 1994). Among the enzymes induced by jasmonate there are lipoxygenases (Bell and Mullet 1991, Melan et al. 1993, Feussner et al. 1995) and the alkaloid-synthesizing enzymes of elicitated cell suspension cultures (Dittrich et al. 1992, Gundlach et al. 1992).

In barley leaves JA exerts two different processes: (i) a down-regulation of mainly plastid proteins such as ribulose bisphosphate carboxylase/oxygenase caused by a translational control (Reinbothe et al. 1993), (ii) synthesis of abundant proteins, so-called jasmonate-induced proteins (JIPs) of different molecular masses as 6, 23, 37, 66, 68, 100 kDa (JIP-6, JIP-23, JIP-37, JIP-66, JIP-68, JIP-100) (Weidhase et al. 1987, Hause et al. 1994). On the basis of sequence homology, JIP-6 was identified as a leaf thionin (Andresen et al. 1992) and JIP-60, which could be shown to be identical with JIP-66 (Demus, unpublished), was found to be a ribosome inactivating protein (RIP) (Chaudhry et al. 1994, Reinbothe et al. 1994). In contrast, no indication of function could be drawn from sequence comparisons in the data bases for cDNA clones coding for JIP-23 (Andresen et al. 1992) or JIP-37 (Leopold et al. 1996). Another group of JIPs in barley was immunologically characterized as a family of lipoxygenases (Feussner et al. 1995).

All these proteins are synthesized upon exogenous application of jasmonate or its endogenous rise, which occurs due to environmental stress, e.g. desiccation or osmotic stress (Lehmann et al. 1995). In any case of JIP gene expression observed so far in barley leaves, jasmonate was found to be the most effective inducer (Wasternack et al. 1995). The question remains, as to whether JIP gene expression can be developmentally, regardless of the JA content of the respective tissue. As known for a number of so-called stress related proteins, these are expressed during distinct developmental stages of a plant life. As described for heat shock

Abbreviations: BCIP, 5-bromo-4-chloro-3-indolyl phosphate; BSA, bovine serum albumine; DIG, digoxigenine; FITC, fluorescein-5-isothiocyanate; JA, jasmonic acid; JIP, jasmonate-induced protein; JM, jasmonic acid methyl ester; NBT, nitroblue tetrazolium chloride; PBS, phosphate buffered saline; PEG, polyethylene glycol; SSC, sodium salt citrate.

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proteins, gene expression is differentially regulated during embryogenesis (Domoney et al. 1991, DeRocher and Vierling 1995, Zur Nieden et al. 1995) as well as during morphogenesis (Duck et al. 1989). Some of the pathogenesis-related (PR) proteins, which are mainly expressed in response to pathogenic attack, are constitutively expressed like β-1,3-glucanases in different tissues of unstressed potato (Garcia-Garcia et al. 1994), chitinases in reproductive organs of rice (Zhu et al. 1993) as well as during somatic embryogenesis in carrot (De Jong et al. 1992) or PR10 in the stigma of potato plants (Constabel and Brisson 1995).

These data prompted us to investigate developmental and tissue-specific expression of JIP-23, the most abundant jasmonate responsive protein of barley leaves. Using immunocytochemical techniques and in situ hybridization, cell- and tissue-specific expression in 6 days old plants was determined. By Western and Northern blot analysis, expression of JIP-23 gene(s) during germination and seedling growth was recorded in comparison with the jasmonate content of the respective tissues.

Materials and Methods

Plant material—Barley (*Hordeum vulgare* cv. Salome) grains were germinated on wet filter paper in the dark at 24°C up to 3 days. At selected time periods, seedlings were dissected, frozen in liquid nitrogen and used for isolation of total RNA and protein as well as for the extraction of endogenous jasmonates, or they were used directly for the histochemical analyses. Light grown seedlings of barley were grown for 6 days in soil under greenhouse conditions in continuous light (300 μE m^{-2}) at 24°C and 60% relative humidity. Treatments with the racemic (±)-jasmonic acid methyl ester (Firmenich Comp., Geneva, Switzerland) was performed by flotation of five cm-segments (cut 1 cm below the tip) of primary leaves of seven days old seedlings on a 45 μM solution (freshly prepared) in a petri dish under continuous light (120 μE m^{-2} s^{-1}) at 25°C.

Extraction of RNA and proteins—Total RNA and protein were isolated simultaneously from each sample. The dissected seedlings as well as the various tissues of the light grown seedlings were homogenized in liquid nitrogen and extracted by Tris-EDTA-buffered phenol. RNAs were precipitated by addition of isopropanol to the aqueous phase (modified according to Chirgwin et al. 1979). Proteins were isolated from the phenolic phase by precipitation with methanol/ammonium acetate according to Meyer et al. (1988).

RNA electrophoresis and Northern blot analysis—RNA electrophoresis (10 μg total RNA per lane), and Northern blot analysis with the cDNA probe pHvJ3015, which codes for JIP-23 (Andresen et al. 1992), were carried out according to Sambrook et al. (1989). Plasmid DNA of pHvJ3015 was isolated using the QIAGEN Plasmid Kit (QIAGEN). The DIG labeling of the insert of pHvJ3015 as well as the hybridization at 65°C were performed according to the supplier's instructions (Boehringer, Mannheim, Germany).

Protein electrophoresis and Western blot analysis—Precipitated proteins (see above) were solubilized in a sodium dodecyl sulphate (SDS) containing buffer and were then separated by SDS polyacrylamide gel electrophoresis (12% polyacrylamide; Laemmli 1970). Two-dimensional separations included isoelectric focussing gels (Weidhase et al. 1987) in the first dimension in combination with SDS-polyacrylamide gels (12%) in the second dimension. After transfer of proteins onto nitrocellulose BA85 (Schleicher & Schüll, Darmstadt, Germany), immunological detection of JIP-23 was carried out with a rabbit polyclonal monospecific antibody raised against the most abundant isoform of JIP-23 of JM-treated barley leaves (arrow in Fig. 2c). The antibody was used at a dilution of 1 : 800 in the Western Light™ Chemiluminescent Detection System (Tropix Inc. Bedford, MA, U.S.A.) according to the supplier's instructions.

Extraction and analysis of endogenous jasmonates—(−)-Jasmonic acid (JA) was extracted, methylated and determined from the dissected seedlings as well as from the various tissues of the light grown seedlings as described by Wasternack et al. (1994). The enzyme immunoassay used followed the protocol of Weiler (1986). The properties of the antiserum are described by Knöfel et al. (1990). The assay is highly specific for (−)-JM and the amino acid conjugates of (−)-JA. The measuring range of standard curves lies between 0.05 and 25 pmol of (−)-JA with a reliable detection limit of 0.1 pmol. Although the detection limit for this assay lies at least three orders of magnitude lower than the average amount found in seedlings, care has been taken with respect to fresh weight determination. By collecting more than 60 different samples per time point or organ, we tried to minimize biological differences as well as deviations of fresh weight. Data of Fig. 1c and 3d were obtained by three independent experiments, each of them done in duplicates. Data of one representative experiment are presented.

Osmolytic analysis of different organs—To determine osmolytic values of different organs during germination, roots, leaves, mesocotyl and scutellum were collected at 14, 25, 39, 49 and 57 h of germination or were taken from 6 days old light grown seedlings. The tissues immediately frozen in liquid nitrogen were processed by three subsequently performed freeze-thaw steps followed by centrifugation at 20,000 × *g* for 30 min using a nylone sieve (10 μm) to collect the cell sap. At least 25 μl of cell sap were used directly or after appropriate dilution for measurement of osmolytic value with a cryoscopic osmometer from Roebling (Berlin, Germany).

Preparation of material for microscopical analyses—Microscopical analysis was performed with (i) seedlings, dissected 48 h after imbibition and (ii) with tissue around the scutellar nodule of 6 days old light grown seedlings (see Fig. 4). Equivalent tissues were fixed with 3% paraformaldehyde in phosphate buffered saline (PBS), supplied with 0.1% Triton X-100 for 2 h at room temperature. After dehydration by graded series of ethanol, samples were embedded in polyethylene glycol (PEG, PEG 1500 : PEG 4000 = 2 : 1) and cut into 5 μm sections (in situ hybridization) or into 2 μm sections (immunolabelling) according to Van Lammeren et al. (1985).

In situ hybridization—Longitudinal as well as cross sections were mounted on poly-L-lysine coated slides, rinsed in 0.01 M Tris-HCl, pH 8.0, and incubated in 1% bovine serum albumine (BSA) in the same buffer for 1 h. The sections were acetylated by incubation with 0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0, for 10 min and washed briefly in 2 × sodium salt citrate (SSC). Prehybridization was performed for 1 h at 45°C in the hybridization buffer [50% formamide, 4 × SSC, 150 μg ml^{-1} tRNA, 0.5% blocking reagent (Boehringer, Germany), 40 units ml^{-1} of RNase inhibitor (Boehringer, Germany)]. Hybridization was carried out with the digoxigenine (DIG)-labelled insert of pHvJ3015

(see above) in the same buffer at 45°C for 16 h. After hybridization washes were performed at 45°C firstly with 50% formamide in 4 × SSC for 10 min, 4 × SSC for 10 min, and finally with 0.1 × SSC for 5 min. Slides were immunolabelled with an anti-DIG-fab-fragment conjugated with alkaline phosphatase (Boehringer, Germany) according to the supplier's protocol. The staining procedure was performed with nitro blue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP). Finally, the slides were enclosed with glycergen (DAKO, Germany) and analyzed by bright field microscopy and differential interference contrast microscopy with a Zeiss "Axioplan" microscope (Zeiss, Germany). Controls done by omitting the DNA probe revealed no signal.

Immunocytochemistry—Sections of the PEG-embedded material were collected on poly-L-lysine coated slides, rinsed with PBS and then incubated with 1% BSA in PBS for 30 min. For immunolabelling rabbit polyclonal antibodies raised against JIP-23 were used (see above) in a dilution of 1 : 400, and the V-type H^+-ATPase-holoenzyme from *Kalanchoë daigremontiana*, in a dilution of 1 : 1,000, respectively. The anti-H^+-ATPase antibody was kindly provided by Prof. U. Lüttge, Darmstadt (cf. Haschke et al. 1989). Goat anti-rabbit-IgG-fluorescein-5-isothiocyanate (FITC) conjugate (Sigma) was used as secondary antibody. After immunolabelling, sections were mounted in para-phenylendiamine (0.2% in glycerol). Control experiments were performed with preimmuneserum.

The staining of sections with aniline blue was done according to Gerlach (1984). The fluorescence of the immunolabelled JIP-23 and H^+-ATPase as well as of callose, stained by aniline blue, was visualized with a Zeiss "Axioplan" epifluorescence microscope using the filter combination: BP 485/20//FT510//LP520.

Results

Expression of the JIP-23 gene(s) during germination of barley seeds—Northern blot analysis for JIP-23 during seed germination revealed first transcript accumulation at 14 h and a transient rise which peaked sharply at 32 h (Fig. 1a). The different amounts of mRNA of seedlings 32 h after imbibition, and of leaves JM-treated for 24 h were measured by densitometric tracing of the hybridization signals (data not shown). The amount of JIP-23 transcript of the seedlings was found to be approximately 10% of the total amount of transcript accumulated by JM-treated leaves. Western blot analysis was used to investigate the occurrence of JIP-23. Figure 1b depicts the appearance of JIP-23 following 25 h of imbibition. Unlike the course of its respective mRNA, the amount of JIP-23 increased up to 39 h but remained constant up to 57 h.

The pattern of the isoforms of JIP-23 occurring during germination, was analysed by two-dimensional gel electrophoresis followed by immunological detection (Fig. 2). At least six different isoforms were recognized among the polypeptides of JM-treated leaves (Fig. 2c). Three of these isoforms, appearing upon JM treatment, were also detectable in seedlings, germinated for 28 h (arrowheads at Fig. 2a, c). Interestingly, the most basic isoform in seedlings did not occur during JM-treatment of leaf segments (arrow at Fig. 2a). Otherwise, three acidic isoforms appeared only in JM-treated leaves (arrows at Fig. 2c). The identity of isoforms, occurring either in seedlings or of JM-treated leaves, was determined by separating a mixture of the two polypeptide extracts (Fig. 2b).

Summarizing, germinating seedlings revealed the synthesis of several isoforms of JIP-23 without exogenous jasmonate application. This was substantiated by inspection whether or not JIP-23 and its transcript occurred in different organs of 6 days old seedlings (cf. Fig. 3a). The mRNA of JIP-23 was detectable only within three parts of the seedling (Fig. 3b): (i) the tissue around the scutellar

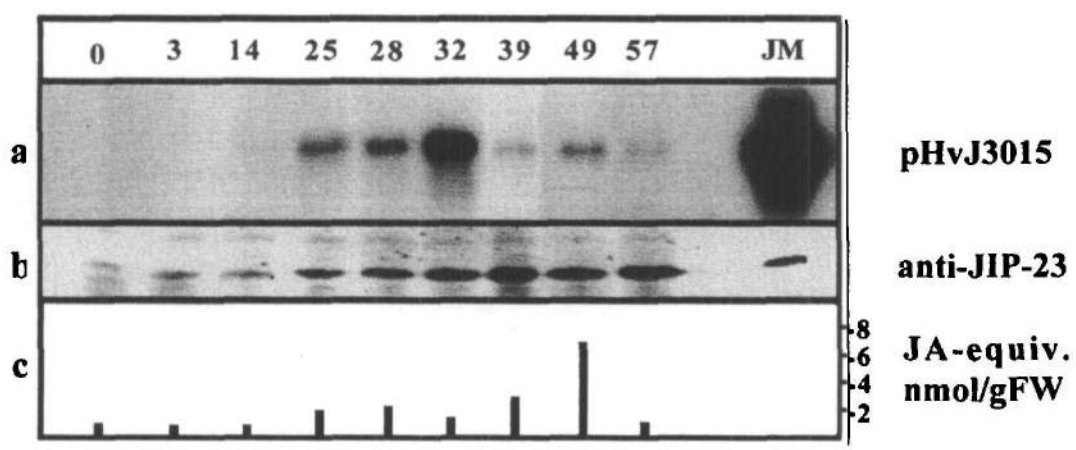


Fig. 1 Accumulation of JIP-23 mRNA (a), JIP-23 (b) and jasmonates (c) in seeds and germinating seeds of barley. Total RNA (10 μg per lane) or total protein (7 μg per lane) were used from different stages of germination. The accumulation of JIP-23 mRNA and JIP-23, respectively, were compared with that occurring in barley leaf segments upon JM treatment. To present the data of JM treatment in a corresponding signal intensity, total RNA (a) and total protein (b) from leaves, 72 h treated with JM, were diluted 1 : 10 before Northern blot and Western blot analyses, respectively. Amount of endogenous jasmonate equivalents (JA-equiv., c) represents the sum of (−)-jasmonic acid methylated after extraction, and its amino acid conjugates. The JA-equivalents are given in nmol per g FW.

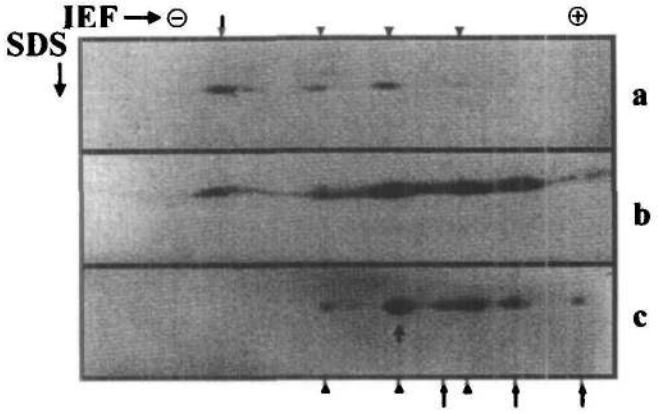


Fig. 2 Isoforms of a 23 kDa protein of developing barley seedlings (a) are partially identical with isoforms of JIP-23 occurring in barley leaves upon JM treatment for 72 h (c). Total protein, in (a) 250 μg and in (c) 25 μg, was separated by two-dimensional gel electrophoresis. Proteins in the area of the 23 kDa isoforms were electroblotted to cellulose nitrate membranes and immunodetected by the Western light ™ Chemiluminescence detection system. The most abundant isoform of JIP-23, marked by an arrow in (c), was originally isolated from JM-treated barley leaves and used for antibody production. At (a), the arrow indicates a developmentally expressed basic isoform not appearing in JM-treated leaves. At (c), acidic isoforms induced exclusively upon JM treatment are marked by arrows, and JM-inducible isoforms being identical to developmentally expressed isoforms in (a) are indicated by arrowheads (also at (a)). To check identity of some isoforms shown in (a) and (c), a mixture of both total protein extracts (250 μg of seedling protein and 25 μg of JM treated leaf protein) was separated and immunodetected in (b).

nodule (mesocotyl), (ii) the scutellum, and (iii) the youngest part of the primary leaf. JIP-23 was found mainly in these three tissues, but a low amount of protein was clearly detectable in roots and the primary leaf, too (Fig. 3c). Moreover, in the seedlings this amount decreased gradually from the scutellar nodule to the tip of the primary leaf.

JIP-23 mRNA is expressed within tissues exhibiting an increased level of endogenous jasmonates—In order to prove whether or not a threshold of endogenous jasmonates is needed to trigger the synthesis of JIP-23, we have analyzed the total amount of jasmonates by an ELISA specific for (−)-jasmonic acid methyl ester and the amino acid conjugates of (−)-JA. Therefore, free (−)-JA extracted from the tissues was methylated. During seed germination the level of jasmonates (Fig. 1c) was found to be higher than that of untreated primary leaves of 7 days old plants (0.6 nmol $(g\ FW)^{-1}$, cf. Lehmann et al. 1995): Between 25 h and 39 h of germination the amount was up to 5-fold and at 49 h of germination about 12-fold higher than that of untreated leaves. Only during the first increase of the endogenous jasmonates an accumulation of JIP-23 mRNA occurred, whereas at 49 h of germination JIP-23 mRNA was hardly detectable.

In 6 days old seedlings a correlation exists for some tissues between the endogenous content of jasmonates and the expression of JIP genes (Fig. 3d versus b). On the one hand, high levels of jasmonates were found in the scutellar nodule, which exhibits JIP-23 mRNA accumulation (lane 2 in Fig. 3b). On the other hand, in the lower part of the primary leaf a remarkable amount of jasmonates appeared, although JIP-23 mRNA was not detectable (lanes 6 and 7 in Fig. 3b versus d). But, the accumulation of JIP-23 protein (lane 6 in Fig. 3c) suggests that its synthesis was already switched on during an earlier stage of development. Within the scutellum the jasmonate content is only slightly higher

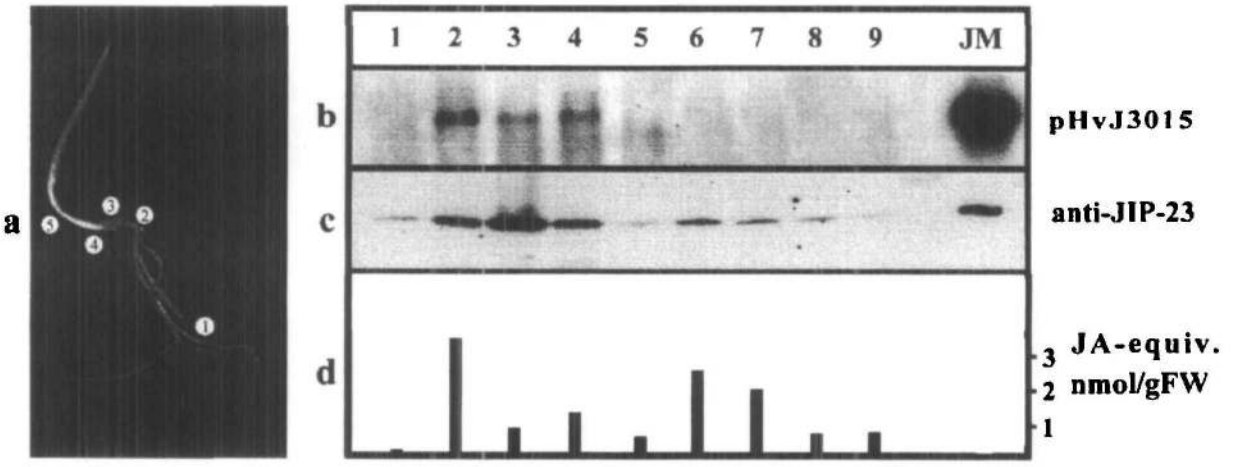


Fig. 3 Accumulation of JIP-23 mRNA (b), JIP-23 (c) and jasmonate content (d) of different organs of a 6 days old barley seedling. The organs are indicated in (a) showing a 3 days old seedling. Total RNA and protein extracts were from roots (1); the transition region between roots and the cotyledons, including the nodal plate of the scutellum (2); scutellum (3); 1 cm of primary leaf below the coleoptile (4); coleoptile (5); or from four 2 cm primary leaf segments starting above the coleoptile (6–9). JM controls are presented identical to that of Fig. 1. Amount of jasmonate equivalents (JA-equiv., d) is given as indicated for Fig. 1.

than that of an unstressed leaf, and the accumulation of JIP-23 mRNA is rather low (lane 3 in Fig. 3). However, the scutellum exhibited high amounts of JIP-23 protein. Since the scutellum contains similarly high amount of jasmonates as the scutellar nodule at 24 h of germination (data not shown), JIP-23 protein formed at this time might be still persisting within the cells after 6 days of seedling development.

Interestingly, the high level of jasmonates coincided with a high osmotic potential in different organs and during germination. Between 14 and 36 h of germination an average osmotic potential of −1.4 MPa occurred in the scutellum, which was clearly higher than that of roots, leaves and mesocotyl (−0.98 MPa). After 24 h of germination there was a continuous decline in the osmotic potential of roots and leaves leading to −0.65 MPa in the primary leaves of 6 days old seedlings, whereas the osmotic potentials of mesocotyl and scutellum remained high, at −1.1 MPa and −1.38 MPa, respectively.

Tissue-specific occurrence of JIP-23 mRNA—The specific occurrence of JIP-23 gene expression in the region of the scutellar nodule of 6 days old seedlings encouraged us to undertake a more detailed inspection by cytological methods. As indicated in the schematic drawing of a 6 days old seedling (Fig. 4), we analysed this region by longitudinal and cross-sections (Fig. 5). We could identify the different tissues within the scutellar nodule: the xylem, the phloem and the parenchymatic cells (Fig. 5b–g) as well as the shoot apex (Fig. 5a).

By in situ hybridization of longitudinal sections (Fig. 5a–c), JIP-23-mRNA became clearly detectable only within the vascular bundles of the scutellar nodule (Fig. 5b, c). The shoot apex of the seedling was free of any label (Fig. 5a). All labelled cells were connected with the phloem. The unlabelled xylem was clearly defined by the typical shape of the tracheids (arrows in Fig. 5c). The phloem-specific occurrence of JIP-23 mRNA was substantiated by in situ hybridizations of cross-sections through the upper and the lower part of the nodal plate of the scutellum (Fig. 5d, e, f, g, for the location of sections within the seedling see Fig. 4). Again, the label was found exclusively within cells connected with the vascular bundle, especially with the phloem. The scutellum as well as the vascular cylinder of the root did not show any label (data not shown).

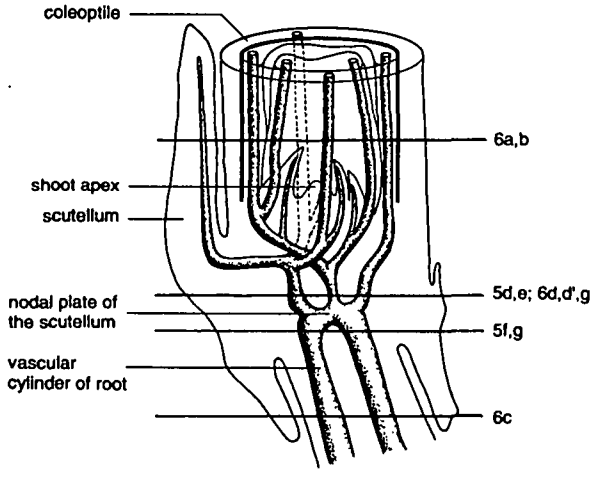


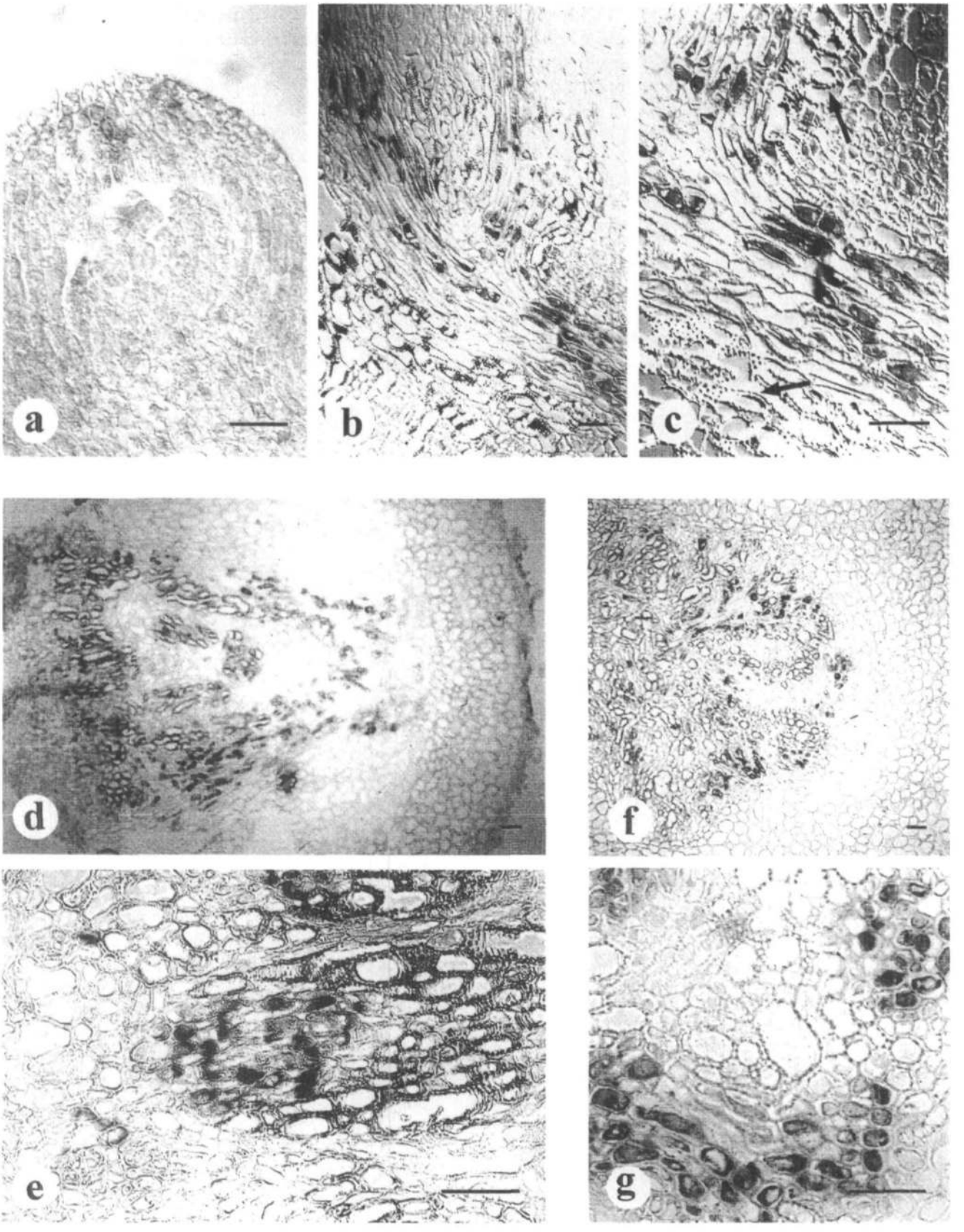
Fig. 4 Schematically longitudinal view of the transition region of a barley seedling (adapted from Esau 1965). The vascular bundles are shown by stippled areas. The positions of cross-sections used for in situ hybridization presented in Fig. 5 or for immunocytochemistry shown in Fig. 6 are indicated by the corresponding numbers and letters used in both figures.

Tissue-specific localization of JIP-23 protein—The cell type-specific expression of the JIP-23 gene(s) was confirmed by localization of JIP-23 via immunocytochemical techniques. In cross-sections of a 6 days old seedling (indicated in Fig. 4) the location of JIP-23 is visualized by the green fluorescence of the FITC-labeled antibody. JIP-23 was located in the vascular bundles of the scutellar nodule (Fig. 6d, d'), in the parenchymatic cells of the scutellum (Fig. 6b) as well as in the vascular cylinder of the root (Fig. 6c). The primary and secondary leaves seemed to be free of JIP-23 (Fig. 6a). The label within the vascular cylinder of the root (Fig. 6c) points to the location of JIP-23 within the phloem. Here, the tissue is characterized by the typical anatomy of an exarch xylem and an alternative arrangement of xylem and phloem. Moreover, immuno-labeling of cross sections through the scutellar nodule of seedlings germinated for 48 h exhibited the occurrence of JIP-23 within the phloem, too (Fig. 5f, f').

In order to determine which of the phloem cells contain JIP-23, specific staining was used (i) for callose by aniline blue, thus visualizing the sieve plates of the sieve tubes, and (ii) for H^+-ATPase to visualize immunologically the companion cells of the sieve tube complex. As shown in Fig. 6e, the sieve tubes are characterized by their typical shape and the presence of the stained sieve plates (arrows in Fig. 6e). It is well established, that companion cells contain a high amount of H^+-ATPase (cf. Michelet and Boutry 1995) and so this cell-type could be defined by its preferential staining with a FITC-labelled-H^+-ATPase antibody (Fig. 6g). Comparing all these cells, which could be labelled by anti-JIP-23 antibodies, by DIG-labelled cDNA coding for JIP-23 and by anti-H^+-ATPase antibodies, it is obvious, that JIP-23 gene expression in 6 days old seedlings is restricted to the companion cells of the vascular bundles of the scutellar nodule.

Discussion

Developmental expression of various stress-related proteins prompted us to investigate, whether or not jasmonate responsive proteins of barley leaves are expressed



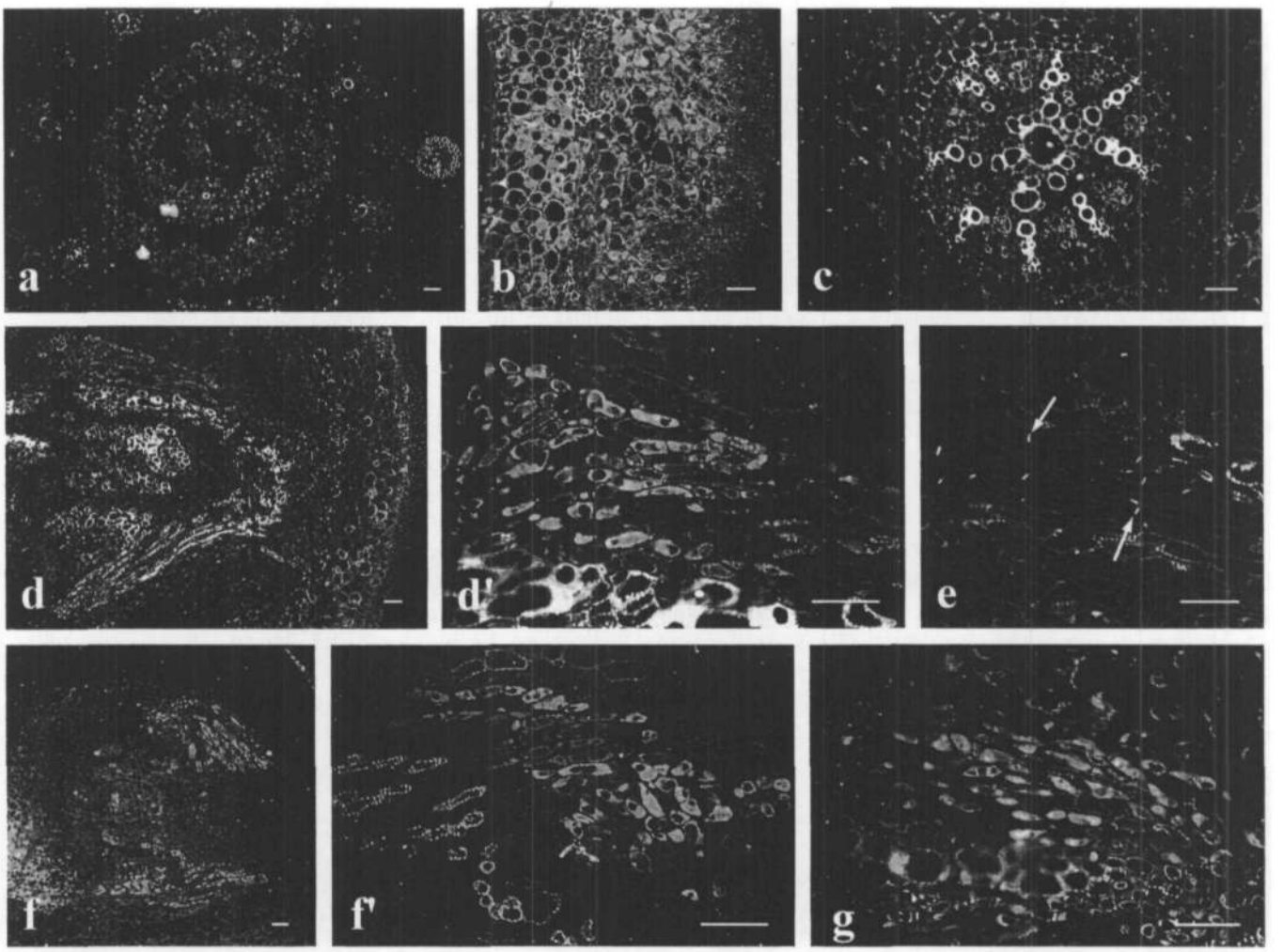


Fig. 6 Survey about the localization of JIP-23 (a)–(d′) as well as H^+-ATPase (g) in 6 days old seedlings and of JIP-23 in seedlings 48 h after imbibition (f, f′) visualized by indirect immunofluorescence with FITC-conjugated antibodies. (a) Cross section through a lower part of the coleoptile and the primary as well as secondary leaf of the seedling. Note the absence of any specific label, the tracheids are visible by the yellow autofluorescence of lignin. (b) Cross section through the scutellum at the same level as in (a) (cf. Fig. 4). The parenchymatic cells are strong labelled as visible by green fluorescence. (c) Cross section of a vascular cylinder of the root. Note the typical anatomy of the exarch xylem and the alternative arrangement of phloem and xylem. Only the phloem exhibits label. (d) Cross section above the nodal plate of the scutellum. The label is restricted to the epidermal layer which is in contact with the endosperm and the vascular bundles. The higher magnification shown in (d′) shows the label within the phloem. By comparison with (e) and (g) the labelled cells can be characterized as companion cells. (e) Parallel section as in (d′) stained with aniline blue to visualize callose. The sieve plates of the sieve tubes are indicated by yellow fluorescence (arrows). (f) Cross section below the nodal plate of the scutellum of a seedling 48 h after imbibition. (f′) shows the higher magnification of (f). Note the comparable distribution of the label as in (d), (d′). (g) Parallel section as in (d′) immunolabelled with antibodies raised against H^+-ATPase to visualize the companion cells. Bars represent 50 μm in all figures.

Fig. 5 Accumulation of JIP-23-mRNA within a 6 days old seedling of barley visualized by in situ hybridization of longitudinal (a)–(c) as well as cross-sections (d)–(g). (a) Longitudinal section through the shoot apex. The cytoplasm-rich cells are unlabelled. (b) Longitudinal section through the nodal plate of the scutellum. Note the label within cells related to the vascular bundles. (c) Higher magnification of (b). The occurrence of JIP-23 mRNA is restricted to cells of the phloem. The xylem is characterized by the typical shape of the tracheids (arrows). (d) Cross section above the nodal plate of the scutellum. Note the label within the regions of the vascular bundles. (e) Higher magnification of a section from the same region as in (d). The label is obviously visible within cells of the phloem. (f) Cross section below the nodal plate of the scutellum. An area of the transition from the endarch to the exarch xylem is visible. (g) Higher magnification of a section from the same region as in (f). The label is visible within the cytoplasm of the phloem-related cells. Bars represent 50 μm in all figures.

developmentally regulated during germination as well as growth of young seedlings. JIP-23, the most abundant JIP of barley, is known to be expressed in leaves upon treatment with exogenous jasmonate or various stresses (Lehmann et al. 1995). To date, there is no suggestion of function by sequence homology (Andresen et al. 1992) or specific expression pattern.

Interestingly, JIP-23 is synthesized upon JM treatment in several immunological related isoforms. During germination this pattern of JIP-23 isoforms is altered in comparison with that of JM-treated leaves (Fig. 2a versus c) by the appearance of a newly synthesized basic isoform and disappearance of acidic isoforms. But it is unclear, whether these polypeptides are distinct gene products or whether they are post-transcriptionally or post-translationally modified forms. Preliminary studies gave no indications for post-translational modifications such as phosphorylation or glycosylation (unpublished results). Southern blot analysis revealed the presence of at least two different genes for JIP-23 (unpublished results). If two genes exist indeed, and if both of them have a function, then they are indistinguishable in the size of their transcripts (Fig. 1, 3). Possible minor differences in the nucleotide sequences among the JIP-23 genes may result in the different pattern of isoforms. Nevertheless, it is noticeable, that at least three isoforms, which appear upon JM treatment (Fig. 1c), are identical to those occurring upon osmotic stress by sorbitol treatment (Lehmann et al. 1995, Wasternack et al. 1995) or during germination (Fig. 1a). This may suggest a similar function of the proteins, both in response to stress and in the germination process.

In a wide range of conditions tested so far (Wasternack et al. 1994, 1995, Lehmann et al. 1995) JIP-23 was synthesized only in response of a preceding rise of endogenous jasmonates. Therefore, the developmental expression of JIP-23 was tested parallel with the determination of jasmonates. During seed germination a specific appearance of JIP-23 transcript after 25 h was weakly correlated with an enhanced content of jasmonates in the whole seedling (2.5 nmol $(g\ FW)^{-1}$) (Fig. 1). This amount is nearly in the range as that found for stressed leaves (4 nmol $(g\ FW)^{-1}$ in 24 h sorbitol treated leaves, Wasternack et al. 1995) capable to induce JIP-23 gene expression (Lehmann et al. 1995). Compared to non-stressed leaf segments of 7 days old seedlings, which contain 0.6 nmol jasmonates $(g\ FW)^{-1}$ (Lehmann et al. 1995), the level determined at 25 h of germination might function as threshold level able to switch on JIP-23 gene expression. However, a second increase of the amount in endogenous jasmonates, e.g. at 49 h of germination, did not induce the expression of JIP-23 (Fig. 1), suggesting an altered threshold necessary for the induction or a sequestration of jasmonates as proposed recently for transgenic potato overexpressing an enzyme of the biosynthetic pathway of JA (Harms et al. 1995).

In 6 days old seedlings only in the scutellar nodule the accumulation of JIP-23 mRNA coincides with an increased level of jasmonates compared to unstressed leaf segments (Fig. 3, lane 2). However, remarkable accumulation of JIP-23 in the scutellum lacking high jasmonate content (Fig. 3, lane 3) or in the lower parts of the primary leaf lacking JIP-23 mRNA (Fig. 3, lanes 4 and 6) may indicate persistance of JIP-23 from a preceding JIP-23 gene expression. Indeed, after 24 h of germination high amount of jasmonates in the scutellum coincides with JIP-23 mRNA accumulation (Fig. 1a). Once synthesized JIP-23 might occur in the parenchymatic cells of the scutellum due to its negligible turnover known for barley leaf segments (Böhling, pers. communication).

The scutellum is known to challenge a tremendous solute transport from the endosperm into the growing seedling. In a more specific manner, companion cells being the physiologically active part of the sieve tube complex of the phloem, function in feeding the neighbouring cells/tissues. Preferentially those cells, which were found to synthesize JIP-23 (Fig. 6), are highly stressed osmotically via carbohydrate breakdown products (cf. Thorne 1985, Briggs 1992). Thus, a speculative scheme can be envisaged: Cells which are highly stressed during a distinct stage of development may respond with jasmonate synthesis, which enable them to form proteins like JIP-23. Indeed, at 24 h of germination the scutellum showed the high osmolytic value of -1.4 MPa and a high jasmonate level, both of them corresponding to values found in osmotically stressed leaves (Lehmann et al. 1995). The detailed function of JIP-23 is unknown. But the concomitant occurrence of cellular stress with the synthesis of JIP-23 up to its abundant occurrence suggest possible protective effects of JIP-23 on cell constituents during stress periods. Whether these effects may occur directly by protein-protein-interactions or indirectly by preserving cell water or by another mechanism, remains to be answered.

Although we cannot exclude, that a developmentally regulated JIP gene expression exists separately, the data presented here and published elsewhere (Lehmann et al. 1995) indicate jasmonate to be the ultimate signal in turning on expression of certain genes upon environmentally or developmentally caused stress.

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