Changes in the Polypeptide Patterns of Barley Seedlings Exposed to Jasmonic Acid and Salinity

Liliana Todorova Maslenkova, Tania Simeonova Miteva, and Losanka P. Popova*

Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Building 21, Sofia 1113, Bulgaria

ABSTRACT

Soluble and thylakoid membrane proteins of jasmonic acid (JA)-treated and salt-stressed barley (Hordeum vulgare L.) seedlings were investigated using 15% sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis. High JA concentrations induced marked quantitative and qualitative changes in polypeptide profiles concerning most of the proteins with approximately equal mobility, as in NaCl-stressed plants. The most obvious increase in thylakoid polypeptide band intensity was at 55 to 77 kilodaltons (kD). The relative size of some polypeptides with apparent molecular masses above 66 kD and of polypeptides with lower molecular masses in the region of 20.5 to 15 kD was enhanced. At the same time, one new band at 31 to 31.5 kD was well expressed at 25 and 250 micromolar JA concentrations and became discernible in the 100 micromolar NaCl-stressed plants. The intensity of some polypeptides of soluble proteins (molecular masses of 60, 47, 37, 30, and 23.4 kD) increased with increasing JA concentration, whereas the intensities of other polypeptide bands (55, 21.4, and 15 kD) decreased. Enhanced levels of 60-, 47-34-, and 30-kD polypeptides and reduced levels of 55- and 15-kD polypeptides were present in NaCl-treated plants. The appearance of one new polypeptide, of 25.1 kD, was observed only in NaCl-treated plants. At 100 millimolar NaCl, an eightfold increase in proline content was observed while at 250 micromolar JA, the proline content was threefold over the control. It is hypothesized that exogenously applied jasmonates act as stress agents. As such, they provoke alterations in the proline content and they can modulate typical stress responses by induction of stress proteins.

During their growth and development, plants are subjected to a variety of environmental stresses such as heat, drought, cold, salt, and anaerobiosis. One approach to understanding the ability of plants to tolerate environmental stresses is to identify stress-induced changes in the levels of individual proteins, with the assumption that adaptation to stress is the result of altered gene expression (15).

Changes in proteins (from inhibition of synthesis of a great number of proteins to the induction of synthesis of new sets of proteins) can result from a variety of environmental stresses such as heat shock (16), anaerobiosis (34), water stress (10), cold acclimation (7), and salt stress (11).

Two specific plant reactions to stress are the accumulation of high levels of ABA and proline. ABA accumulates in leaves of most plants during water stress (14), high and low temperature stress (9), and salt stress (37). The suggestion has been made that the production of ABA is a common response to a variety of environmental stresses (9).

Although several modes of action of the so-called classic phytohormones (auxins, cytokinins, gibberellins, ABA, and ethylene) and some putative plant growth regulators such as polyamines and jasmonates are hypothesized, the general opinion points to their primary actions at the level of gene expression. Applied exogenously, these substances can induce physiological changes identical with characteristic parts of the stress responses (4, 25, 26). Farmer and Ryan (12) demonstrated that a highly sensitive mechanism is present in Solanaceae and Fabaceae families that can activate plant-defensive genes in response to volatile methyljasmonate.

Plant physiological responses to environmental stresses result in slower growth and photosynthetic rate, leading finally to yield reduction. To a certain extent, the action of ABA is similar to the effect of JA, its methyl ester (JA-Me), and salt stress on a number of photosynthetic parameters. Exogenous treatment of barley seedlings with ABA and JA reduces the rate of photosynthetic CO$_2$ fixation and the activity of ribulose biphosphate carboxylase increases the rate of photospiration, and increases both the CO$_2$ compensation point and stomatal resistance (28, 29). ABA and JA applications at concentrations of 25 and 250 μM, respectively, lead simultaneously to changes of photosynthetic light reactions connected with chloroplast thylakoid membranes. Maslenkova et al. (20, 21) have established inhibition of the Hill reaction activity and some changes in the kinetic characteristics of the flash-induced O$_2$ evolution, which probably are determined by changes in the chloroplast membrane structure during a prolonged treatment with ABA and JA.

Both substances induce synthesis and accumulation of several abundant and specific polypeptides. ABA- and JA-induced polypeptides are identical, and the assumption is that their synthesis takes place on cytoplasmic ribosomes (37). The action of jasmonates resembles certain aspects of ABA action, but differs in other aspects, e.g. JA-Me is unable to replace ABA for the induction of α-amylase or other enzymes in barley aleurone layers (24).

In this work, we have investigated the effect of exogenous

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2 Abbreviations: JA, jasmonic acid; CA, carbonic anhydrase; JA-Me, methyl ester of jasmonic acid; LSU, large subunit of Rubisco; SSU, small subunit of Rubisco.
application of JA and increasing NaCl concentrations on the polypeptide composition of soluble and thylakoid membrane proteins. We are interested in specific polypeptide changes, characteristic for salt and JA treatments, that eventually could be related to determining functional characteristics.

The assumption is that exogenously applied jasmonates, as presumed stressors, provoke alterations in the proline content and can modulate typical stress responses by induction of stress protein.

MATERIALS AND METHODS

Plant Material

Seeds of barley (Hordeum vulgare L., var Alfa) were germinated for 2 d in two layers of moist filter paper in moist vermiculite at 25°C in the dark. Then they were transferred into Petri dishes containing 40 mL distilled water or equal amounts of water solution from the required JA and NaCl concentrations (2.5–250 μM JA and 25–100 mM NaCl). The solutions were changed every 24 h. During the experimental period, the seedlings grew in a growth chamber under white fluorescent lamps (35 W·m⁻²), with 12-h light and dark periods. RH was about 50%.

Protein Extraction

Second-leaf tissues of 7-day-old seedlings were harvested. One g of leaf tissue without the major veins was ground in a mortar on ice with 5 mL extraction buffer containing 0.33 M sorbitol, 0.05 M Hepes-NaOH, 0.002 M KNO₃, 0.002 M EDTA, 0.001 M MnCl₂, 0.001 M MgCl₂, 0.0005 M K₂HPO₄, 0.02 M NaCl, and 0.2 M Na-isoascorbate, pH 7.6. The homogenate was filtered through four layers of cheesecloth and centrifuged at 10,000g for 20 min.

Thylakoid Membrane Preparation

Barley thylakoid membranes were isolated as described by Camm and Green (6) and were suspended in a medium containing 0.4 M sucrose, 10 mM NaCl, 5 mM MgCl₂, and 40 mM Hepes-NaOH, pH 7.5.

Gel Electrophoresis

One-dimensional gel electrophoresis was performed according to the procedure of Laemmli (17) on 15% (w/v) acrylamide slab gels (1.5-mm thick), containing 0.1% (w/v) SDS and 375 mM Tris-HCl, pH 8.7. Samples of soluble and membrane fractions were solubilized in a sample buffer containing 62.5 mM Tris-HCl (pH 6.8), 2% (w/v) SDS, 5% (v/v) β-mercaptoethanol, and 10% (v/v) glycerol. Thylakoid membranes were incubated in solubilizing buffer for 30 min at room temperature (SDS/Chl, 20/1). Protein corresponding to 10 to 15 μg Chl was applied to each lane. Samples of soluble fractions containing 60 μg protein were boiled for 3 min in sample buffer and loaded on the gels. After electrophoresis, gels were stained with 0.2% (w/v) Coomassie brilliant blue R-250 in methanol/acetic acid/water (4/1/5, v/v). Destaining was carried out in methanol/acetic acid/water (4/1/5, v/v). The dried gels were scanned at 560 nm using Shimadzu CS-930 TLC. Mol wts were estimated from a standard plot using lysozyme (14,300), β-lactoglobulin (18,400), trypsinogen (24,000), CA (30,000), ovalbumin (43,000), BSA (66,000), and phosphorylase A (94,000).

Protein Determination

The soluble protein content was determined by the method of Lowry et al. (19), with BSA as the standard.

Proline Determination

The proline concentration was determined spectrophotometrically at 520 nm after Bates et al. (3).

Chemicals

±JA was a generous gift from Professor Sembdner of the Institute of Plant Biochemistry, Halle, Germany. Other chemicals used were of the highest grade of purity.

RESULTS

Changes in Polypeptide Composition of Chloroplast Thylakoid Membranes from Barley Seedlings Treated with JA and NaCl

SDS-PAGE resolved more than 20 polypeptide bands (Fig. 1, A and B) in thylakoids isolated from control barley plants.

![Figure 1. Polypeptide profiles of thylakoid membranes isolated from control barley plants (lanes 1 and 5), from plants treated (A) with 2.5 μM JA (lane 2), 25 μM JA (lane 3), 250 μM JA (lane 4), and from plants treated (B) with 25 μM NaCl (lane 6), 50 μM NaCl (lane 7), and 100 μM NaCl (lane 8). Proteins corresponding to 15 μg Chl were subjected to electrophoresis. □. Proteins increased in thylakoids of treated plants. Arrowhead denotes the position of 31- to 31.5-kD protein calculated from M₅ standard curve. Molecular masses of marker proteins are given in kD.](https://www.plantphysiol.org/)
and from plants treated with different JA and NaCl concentrations. The treatment with 25 and 250 μM JA resulted in several quantitative and qualitative changes in polypeptide profiles. Since equal amounts of Chl are represented in each lane, the data clearly demonstrate that the changes in the polypeptide bands are not accompanied by any changes in the amount of free Chl, i.e. pigment-protein complexes are equally subjected to denaturation. Changes are reproducible in individual experiments and, considering the insignificant diminution of Chl content in the leaves at higher JA concentrations, the qualitative changes in the individual polypeptide bands can be considered as reliable. As compared to the control, JA-treated plants (Figs. 1A and 2) show the following changes: (a) increasing JA concentrations lead to appreciable changes in the intensity of Coomassie-stained bands at 55 to 57 kD. The intensity of some bands above 66 kD and of some bands with a lower molecular mass in the region of 20.5 to 15 kD slightly increased. Among them, the relative share of the bands at 16.9 and 17.5 kD was the most obvious; (b) some polypeptides migrating with apparent molecular masses of 23.5 to 24 kD and 59 to 62 kD may be slightly decreased in plants treated with high JA concentrations; (c) SDS-PAGE shows the appearance of a new band with an apparent molecular mass of 31 to 31.5 kD that correlates with the observed alterations in the 26- to 27-kD band.

Figure 1B shows that a 7-d treatment of barley seedlings with 50 or 100 mM NaCl leads to characteristic alterations in the electrophoresis profiles of the thylakoid membranes, too. A comparison of the bands in Figures 1, 2, and 3 clearly shows that these changes concern mainly the proteins with approximately equal mobility. The most prominent polypeptide band had a molecular mass of 56 to 57 kD. The relative share of polypeptides with apparent molecular masses of 17.5, 16.2, and 15.5 kD increased at high NaCl concentrations. At the same time, one band at 31 to 32 kD was well expressed in JA-treated plants and became slightly discernible in the samples from 100 mM NaCl-treated plants.

Changes in Polypeptide Composition of Soluble Proteins from Barley Seedlings Treated with JA and NaCl

Polypeptide patterns of the SDS-extractable proteins from the soluble fractions (supernatants after 10,000g) are given in Figure 4. About 30 or 31 polypeptide bands with molecular masses from 98 to 14.3 kD were resolved. The major polypeptide bands in the control have molecular masses of 60, 55, 37, 32, 27.5, 21.4, and 15 kD. As compared to the control, JA-treated variants show the following changes: (a) increasing JA concentrations lead to a drastic reduction in the level of 55- and 15-kD polypeptides corresponding, respectively, to the LSU and SSU of Rubisco. The positions of both subunits of Rubisco were identified by running purified barley Rubisco as a marker. Coomassie blue-stained gels were scanned at 560 nm to quantify separated polypeptides (Table I). The quantity of LSU declined from 35.8% at the control to 7.2% at 250 μM JA. For SSU this percentage becomes 7.9 at the control and 2.6 at 25 μM JA. (b) The quantity of several polypeptides increased with the level of JA concentration—their relative molecular masses were found to correspond to 60, 47, 37, 30, and 23.4 kD. (c) One polypeptide with molecular mass 21.4

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**Figure 2.** Densitograms of 15% SDS-polyacrylamide slab gel showing Coomassie-stained thylakoid polypeptides: a, 250 μM JA; b, 25 μM JA; c, 2.5 μM JA; d, control (H2O). Vertical arrows indicate the molecular mass of polypeptide bands in kD. The horizontal arrow denotes the direction of band migration.
kD, well expressed in the control, was greatly decreased in JA-treated seedlings. (d) Among the polypeptides with higher molecular mass, two polypeptides, i.e., 74 and 76 kD, increased in the treated plants. Some other bands with higher molecular mass also increased; however, the resolution capacity of the gel did not allow us to obtain a better separation.

Figure 5 shows SDS-extractable proteins from the control-soluble fraction and from NaCl-grown seedlings. The major polypeptide bands are: 60, 55, 37, 34, 26.3, 21.4, and 15.1 kD. Two bands with molecular masses of 76 and 78 kD from the high molecular polypeptides are prominent, and some very faint bands with molecular masses between 120 and 105 kD were not well separated on the gel. The main differences established between control and NaCl-treated seedlings were: (a) 76-, 60-, 47-, 34-, and 30-kD bands are more intensive in NaCl treatments; (b) with an increasing NaCl concentration, the level of 55- and 15-kD polypeptides, corresponding to LSU and SSU of Rubisco, was strongly decreased. Their percentage content declined in the control from 37 for LSU
Table I. Major Polypeptide Differences between the Control and JA-Grown Plants

<table>
<thead>
<tr>
<th>Polypeptide Band Mol Wt</th>
<th>Jasmonic Acid-Grown Plants</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>kD</td>
<td>%</td>
</tr>
<tr>
<td>60.0</td>
<td>3.9*</td>
</tr>
<tr>
<td>55.0</td>
<td>35.8</td>
</tr>
<tr>
<td>47.0</td>
<td>3.8</td>
</tr>
<tr>
<td>45.0</td>
<td>3.8</td>
</tr>
<tr>
<td>37.0</td>
<td>5.0</td>
</tr>
<tr>
<td>34.0</td>
<td>6.8</td>
</tr>
<tr>
<td>32.0</td>
<td>6.3</td>
</tr>
<tr>
<td>30.0</td>
<td>2.8</td>
</tr>
<tr>
<td>27.5</td>
<td>4.9</td>
</tr>
<tr>
<td>23.4</td>
<td>3.7</td>
</tr>
<tr>
<td>21.4</td>
<td>3.7</td>
</tr>
<tr>
<td>15.0</td>
<td>7.9</td>
</tr>
</tbody>
</table>

*Values indicate percentage of the total extractable proteins at stationary phase based on the scanning of Coomassie blue-stained gels.

...down to 13 in the plants treated with 100 mM NaCl. The decrease in SSU Rubisco level was drastic (Table II); (c) in NaCl-treated seedlings, a new polypeptide with molecular mass 25.1 kD—not present in the control—was observed; (d) when 25 and 100 mM NaCl was applied, the level of one polypeptide with 18 kD increased.

**JA and NaCl Effect on Proline Content**

Proline content in leaves of barley seedlings grown for 7 d on JA and NaCl was determined (Table III). In the experiments carried out with three levels of NaCl treatments, proline levels were increased with increasing NaCl concentrations. The most prominent effect was at 100 mM NaCl, an eightfold rise as compared to the control. Proline accumulation as a response to salt stress is a well-known phenomenon in many plant species, including barley (5, 36). It was observed in our experiments that the proline content in JA-treated barley seedlings does increase, as does the proline content in seedlings subjected to salt stress. At 250 μM JA, a threefold increase of proline content as compared to the control was observed. The effect of JA established in our study was very similar to the well-known effect of ABA on proline accumulation.

**DISCUSSION**

Although the primary site(s) of JA action is not yet known, some observations point to cell membranes that, very soon after JA-Me treatment, lose integrity and show a disordered ultrastructure (26). It can be assumed that, like other stress factors, the exogenous JA application diminishes chloroplast photosynthetic activity as a result of effects on the thylakoid membranes and light-induced reactions connected with them. Generally, Calvin cycle enzymes involved in CO₂ fixation (ribulose bisphosphate carboxylase) are more resistant. The decrease in the generation of ATP and reducing equivalents play an important role in the regulation of Rubisco. It is
Table II. Major Polypeptide Differences between the Control and NaCl-Grown Plants

<table>
<thead>
<tr>
<th>Polypeptide Band Mol Wt</th>
<th>NaCl-Grown Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>kD</td>
<td>%</td>
</tr>
<tr>
<td>78.0</td>
<td>1.1*</td>
</tr>
<tr>
<td>76.0</td>
<td>3.1</td>
</tr>
<tr>
<td>60.0</td>
<td>5.1</td>
</tr>
<tr>
<td>55.0</td>
<td>37.5</td>
</tr>
<tr>
<td>37.0</td>
<td>?</td>
</tr>
<tr>
<td>34.0</td>
<td>10.3</td>
</tr>
<tr>
<td>30.0</td>
<td>?</td>
</tr>
<tr>
<td>26.3</td>
<td>12.6</td>
</tr>
<tr>
<td>25.1</td>
<td>?</td>
</tr>
<tr>
<td>21.4</td>
<td>6.1</td>
</tr>
<tr>
<td>18.0</td>
<td>3.1</td>
</tr>
<tr>
<td>15.1</td>
<td>3.5</td>
</tr>
</tbody>
</table>

* Values indicate percentage of the total extractable proteins at stationary phase based on the scanning of Coomassie blue-stained gels.

obviously clear that the changes in the electron transport capacity of membranes can provoke a decrease in the effectiveness of light utilization, leading thus to the observed decrease of CO₂ fixation and enzyme activity. Our previous investigations (20–22) show that both salt stress and exogenous application of plant regulators, JA and ABA, inhibit PSII activity proceeding from the inhibition of steady-state O₂ evolution (Hill reaction activity) and flash-induced O₂ evolution pattern alterations. A higher degree of participation of the cooperative mechanism of O₂ evolution connected with the functioning of PSII β-centers in the stroma-situated thylakoids was hypothesized and supported by EM data showing a disruption of the granal structure after prolonged salt stress (18) and exogenous application of ABA (8) and JA (32).

The question arose: Are the observed chloroplast morphological and functional changes in the treated plants accompanied by changes in the thylakoid protein profiles? The analysis of polypeptide pattern obtained at the level of resolution capacity of our SDS-PAGE technique shows that the 7-d treatment with JA and NaCl visibly changes the polypeptide composition of the thylakoid membranes. A certain amount of the change concerns proteins with approximately equal electrophoretic mobility in both stress factors applied. We have observed a substantial enrichment (based on the intensity of Coomassie staining) of one polypeptide with an apparent molecular mass of 55 to 57 kD. Proteins that may contribute to occurrence of bands in this region are, for example, the β-subunit of coupling factor 1 or the antenna Chl a protein complex of PSII core complex apoprotein. We can eliminate the contribution of a large Rubisco subunit because the method applied for the isolation of thylakoid membranes restricts, to a great degree, contamination with this enzyme. Polypeptide profiles show slightly enhanced intensity of the band in the region of Chl-protein complex I apoprotein in 68 to 70 kD and a certain reduction in the 23- to 24-kD band, belonging to the PSII O₂-evolving system.

Polypeptide profiles in JA-treated samples reveal an additional polypeptide migrating in 31 to 32 kD. However, in the region of 30 to 34 kD, there are several polypeptides with very similar mobility, i.e., the so-called D₁ and D₂ polypeptides of PSII reaction center and 32-kD extrinsic polypeptide. Thus, for the determination of the band at 31 to 32 kD, other approaches should be used. It is worthwhile to note that Hayden et al. (13) have observed the appearance of one 31-kD polypeptide that correlates with modification of light-harvesting Chl a/b complex in maize leaves exposed to chilling temperatures. According to these authors, accumulation of this protein could be an important factor for the determination of chill-induced decrease in the PSII photochemistry and carbon assimilation quantum yield (2). The 31-kD polypeptide was immunologically attributed to Chl a/b protein complex tightly associated with PSII, which plays an important role in the mediation of the electron transport from light-harvesting Chl a/b complex to PSII. Similar profile changes after both JA and NaCl treatment are observed in the region of lower molecular masses 20.5 to 15 kD, at which some polypeptides of unknown origin increase quantitatively with the increase of stress factor concentration.

We assume that the differences between the polypeptide profiles reflect some structural differences between the control membranes and those of the treated barley seedlings, and that they may be related to the functional differences in PSII reaction activity and O₂ evolution. The observed changes can refer either to the sensitivity of thylakoid membranes towards the stress or to tolerance, with either requiring the synthesis of new proteins. It is quite clear that further isolation and characterization of chloroplast membrane proteins is needed to allow an unequivocal determination of the origin of these proteins and their roles in photosynthetic reactions under conditions of salinity and JA treatment.

In addition to the well-known role of ATP and NADPH on the rate of photosynthetic CO₂ fixation, the level and the activity of photosynthetic enzymes are of substantial impor-

Table III. Effect of NaCl and JA on the Content of Proline in Barley Leaves

The value of the control for NaCl-treated samples is 1.119 μmol proline/g fresh weight and for JA-treated samples is 1.420 μmol proline/g fresh weight.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Proline Content</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O (C)</td>
<td>129.3 ± 19.6a</td>
<td>100.0</td>
</tr>
<tr>
<td>NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 mM</td>
<td>192.0 ± 27.7</td>
<td>148.84</td>
</tr>
<tr>
<td>50 mM</td>
<td>393.3 ± 6.7b</td>
<td>304.18</td>
</tr>
<tr>
<td>100 mM</td>
<td>1040.0 ± 23.1b</td>
<td>804.33</td>
</tr>
<tr>
<td>H₂O (C)</td>
<td>164.2 ± 6.6</td>
<td>100.0</td>
</tr>
<tr>
<td>JA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 μM</td>
<td>205.1 ± 4.6c</td>
<td>125.00</td>
</tr>
<tr>
<td>25 μM</td>
<td>275.3 ± 3.0d</td>
<td>167.70</td>
</tr>
<tr>
<td>250 μM</td>
<td>480.0 ± 14.5e</td>
<td>292.70</td>
</tr>
</tbody>
</table>

* Values are means ± se for five experiments. ° P < 0.001.
tance for the complete photosynthesis. Our knowledge of the
details of chloroplast protein synthesis during stress is still
insufficient. In this respect, Rubisco synthesis, both under
stress and in response to exogenous application of phytohor-
omones, is relatively much better investigated (30, 31, 38). The
results of our investigations showed that ABA and JA caused
a decrease in the level of total soluble protein, particularly in
the level of Rubisco. Radioactive labeling experiments showed
that both substances inhibited Rubisco synthesis, the influ-
ence of JA, however, being more strongly expressed on the
SSU of Rubisco. Weidhase et al. (39) reported that JA blocked
the activity of cytoplasmic ribosomes and caused inhibition on SSU of Rubisco. We have observed a similar mode of action in barley seedlings treated
with NaCl, which leads us to speculate that NaCl and JA
control the Rubisco synthesis by means of a similar mecha-
nisms (23).

Polypeptide patterns of leaf-soluble proteins in barley seed-
lings treated with JA and NaCl showed some other common
characteristics too, i.e. two polypeptides with molecular
masses 60 and 30 kD exhibited a definite increase. Weidhase et al. (39) and Mueller-Uri et al. (24) demonstrated that the
exogenous application of jasmonates (JA and JA-Me) induced
the appearance of at least three classes of abundant polypep-
tides having molecular masses of 66, 37, 30, and 23 kD. They
argue that these proteins are de novo synthesized and are not
degradation products of abundant proteins—for instance,
those of Rubisco. Such an increase of the level of 37-, 30-, and 23-kD polypeptides has also been established by us in the
same plant species—Hordeum vulgare L., but we did not
observe any induction of 66-kD polypeptide, which is prob-
ably due to differences in experiments and between the plant
varieties.

The function(s) of jasmonate-induced polypeptides is not
known. It was presumed that they participate in the modula-
tion of aging reactions or of the plant physiological responses
to stress (27). In our recent communication (33), we stated
that in plants grown on JA and NaCl, a substantial increase
of the cytoplasmic localized CA was observed (33). We are
tempted to suggest that, under conditions of stress, there may
an induction of CO₂ concentrating mechanism. Insofar as the 30-kD polypeptide observed in this study corresponds to the
same electrophoretic mobility and molecular mass of CA, we
could very cautiously consider the presence of enhanced
synthesis of CA upon stress. To test this hypothesis, further
experiments requiring other techniques including immuno-
chemical have to be carried out.

Differences also have been established in the polypeptide
profiles of seedlings treated with JA and NaCl. Seedlings
treated with JA exhibited more intensive bands of 37 and
23.4 kD, whereas when treated with NaCl, more intensive
bands of 34, 25.1, and 18 kD polypeptides were obtained.
Probably these differences can be regarded as natural signals
belonging to different but partially overlapping stress domains
as proposed by Parthier (27) when comparing ABA and JA
effects on polypeptides.

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