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Gene expression induced by physical impedance in maize roots

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Abstract

Two cDNA clones, *pIIG1* and *pIIG2*, corresponding to mRNAs that accumulate in maize root tips subjected to 10 min of physical impedance, were isolated by differential screening of a cDNA library. The deduced proteins, based on DNA sequence analysis, have molecular masses of 13 and 23 kDa for *pIIG1* and *pIIG2*, respectively. *pIIG1* showed 97% similarity at the nucleic acid level to a maize root cortical cell delineating protein (*pZRP3*) and was also similar to some bimodular proteins that are developmentally or stress regulated in other plant species. *In situ* localization of *pIIG1* showed some expression in cortical cells of control maize roots; however, after a 10 min physical impedance treatment, *pIIG1* accumulation increased greatly in cortical cells and extended to include the procambial region. *pIIG2* did not show sequence similarity with any identified gene of known function, but a bipartite nuclear targeting sequence occurs in its deduced amino acid sequence which indicates it may function in the nucleus. Thus, rapid accumulation of specific mRNAs occurs in maize roots in response to impedance stress, and these mRNAs may be responsible for some responses of the roots to physical impedance.

Introduction

As roots encounter the physical impedance presented by soils, turgor pressure in the root cells increases, the rate of extension growth is reduced and expansion growth is promoted. These rapid changes in root development eventually reduce the plant's ability to absorb water and nutrients by restricting the volume of soil permeated by its roots [29, 35]. Feldman [14] attempted to explain root responses to physical impedance through turgor effects on cell wall extension. However, in experiments in which an external pressure was applied to a solid medium to inhibit root growth, the necessary pressure was less than 1 bar, and thus it was far smaller than the turgor pressure, suggesting that a physical restriction is probably not the primary cause of root responses. Several investigators had previously proposed a hormone-mediated mechanism to explain plant responses to soil compaction [18, 25, 27]. Recent evidence suggests that ethylene plays a critical role in the regulation of responses to physical impedance. Sarquis *et al.* [33] showed that a rapid increase in ethylene biosynthesis occurred when maize seedlings were subjected to physical impedance. Ethylene evolution and ACC and conjugated ACC concentrations all increased by 2- to 3-fold before growth was inhibited. It was also demonstrated [33] that the morphological changes and reduced growth rates resulting from physical impedance were largely reversed by pretreatment of seedlings with a combination of AVG, an ethylene biosynthesis inhibitor [1], and silver ion, an ethylene action inhibitor [6].

We apply physical impedance to roots growing in a membrane-enclosed column of baked clay granules inside of a pressurized cell [33]. The baked clay granules are not compressible, but pressure on the membrane increases the rigidity of the matrix through which the roots elongate. The resistance to growth inhibits elongation and promotes swelling of the root tips only [33],

The nucleotide sequence data reported appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers AF001634 (*pIIG1*) and AF001635 (*pIIG2*).

a response mimicked by unimpeded roots exposed to ethylene. After exposure to impedance, roots show no discoloration, wounding or leaking of fluids under unaided or microscopic visual examination. When the pressure on the column is released, the rate of ethylene production drops rapidly, which is another indication that the experimental system is simulating resistance to growth rather than wounding the roots. In addition, physically impeded roots develop internal air spaces [20], aerenchyma, which are visually identical to aerenchyma which develop in response to low O_2 or transient deficiencies of N and P [12, 13]. The latter treatments do not involve any physical contact, but they, along with physical impedance, increase ethylene production rates [13, 33] or sensitivity to ethylene [20]. Finally, elongation rates of impeded roots are restored to 90% of control rates by pretreatment of seedlings with ethylene biosynthesis and action antagonists, a result illustrating that the fritted clay matrix does not physically limit growth but rather impedes it so as to activate a physiological response. These relationships have led us to conclude that physical impedance of root growth is not simply another case of thigmomorphogenesis or seismomorphogenesis [7, 8, 23, 30, 31]. As will be discussed later, a preliminary screening of maize root mRNAs with cDNA probes for three of the TCH genes [8] produced results consistent with the supposition that impedance of root growth is not a touch or wounding phenomenon.

While changes in root morphology and hormone levels for plants subjected to physical impedance have been documented, the mechanisms that regulate these changes, especially in the early stages of stress exposure, remain unknown. Although ethylene biosynthesis in the root increases within one hour of imposition of impedance stress [22, 33], the early steps in signal transduction which initiate the rapid and specific responses are still unclear. We have differentially screened for genes expressed preferentially in 10 min physically impeded maize roots to elucidate early steps in the signal transduction pathway. In this paper we report the isolation and characterization of two cDNA clones, *pIIG1* and *pIIG2*, which correspond to poly(A)$^+$ RNAs which accumulate after only 10 min exposure to physical impedance.

Materials and methods

Plant growth and treatments

Maize (*Zea mays* L.) cv. TX 5855 seeds were surface sterilized in 1% NaOCl solution for 10 min, rinsed and kept in aerated, distilled water at 25 °C overnight. After germination on moist germination paper for 72 h in the dark at room temperature, seedlings were selected for uniform root length (about 25 mm) and transferred in groups of 8 to 10 into a column of premoistened, fritted clay enclosed in a triaxial cell. All physical impedance experiments were conducted in modified triaxial cells which have been described in detail by Sarquis *et al.* [33]. The triaxial cells allowed application of a controlled gas pressure in the space surrounding a membrane which enclosed the fritted clay growth medium; the pressure increased the rigidity of the clay matrix thereby increasing the physical impedance to growth. After plants were transferred to the triaxial cells, the cells were placed in a growth chamber at 25 °C for 12 h before treatment. Treatments consisted of controls (no pressure) and 100 kPa gas pressure (N_2) applied externally to the membrane enclosed growth medium in the triaxial cells for 10 min. Compressed, humidified breathing air (medical grade) with normal O_2 content flowed through the growth medium while seedlings were enclosed. After treatment, the triaxial cells were rapidly disassembled and 1 cm sections were cut from the root tips, frozen immediately in liquid nitrogen and then stored at −80 °C until extracted.

RNA isolation

Total RNA was isolated from root sections following the method of Chang *et al.* [10]. The poly(A)$^+$ RNA isolation utilized the polyATtract mRNA isolation system (Promega, Madison, WI). The biotinylated oligo(dT) primer was hybridized in 75 mM NaCl + 7.5 mM sodium citrate (0.5× SSC) to the 3′ poly(A) region of the mRNA. Poly(A)$^+$ RNA was isolated by magnetic separation and, the beads were washed several times with 0.1× SSC after which the poly(A)$^+$ RNA was eluted by Rnase-free water.

Construction of maize root cDNA library

The cDNA synthesis reaction was primed with a 50 bp pair oligonucleotide which contained both an *Xho*I restriction enzyme recognition site and an 18 bp poly (dT) sequence (Stratagene, La Jolla, CA), using

poly(A)$^+$ RNA isolated from 1 cm of 10 min impeded root tip sections. *Eco*RI adaptors were ligated to the blunt ends of cDNAs and digested with *Xho*I to release the *Eco*RI adaptor from one end. This method permits the cDNA to be directionally cloned, with the *Eco*RI and *Xho*I 'sticky end', at the 5′ and 3′ ends, respectively. Double-stranded cDNAs were directionally cloned into the commercial bacteriophage λ, Uni-Zap XR (Stratagene) and packaged using lambda *in vitro* Gigapack Gold packaging extracts.

Differential screening of cDNA library and isolation of IIG clones

Differential screening of the cDNA library was carried out to determine if mRNA levels for specific genes changed in response to physical impedance. Single-stranded cDNA probes were prepared from mRNA from maize roots segments exposed to 0 min (control) or 10 min impedance treatment in triaxial cells. Duplicate filters of each NZY (casein enzymatic hydrolyzate and yeast extract) phage plate with a density of ca. 2000 plaques per plate were made according to Maniatis *et al.* [28]. The two cDNA probes were separately hybridized to the duplicate filters. Plaques hybridizing preferentially with cDNA derived from the 10 min stress probe were isolated. After secondary screening, plaques were excised and the plasmids were isolated.

In situ *hybridization*

Root tips 1 cm in length were cut, fixed with 4% formaldehyde and 0.5% glutaldehyde in 100 mM phosphate buffer (pH 7.0) and embedded in paraplast plus (Sigma, St. Louis, MO). Sections (10 μm) were cut with a microtome and placed on poly-L-lysine-coated slides [3]. Because the cDNA was cloned into pBluescript SK+ (Stratagene), either T3 (sense transcript) or T7 (anti-sense transcript) polymerase was used to generate a digoxigenin-labeled RNA probe from the linearized plasmid (Boehringer, Indianopolis, IN). *In situ* analysis was performed with digoxigenin-labeled RNA probe added to the slides and the hybridization temperature was 50 °C overnight. Prehybridization treatment, hybridization conditions and post-hybridization treatments were performed as described by deAlmeida Engler *et al.* [5]. The tissue image and blue color of the resulting signal was visualized by applying alkaline phosphatase-conjugated, anti-digoxigenin antibodies and enzyme substrate. The hybridization signal was then photographed through a Zeiss Photomicroscope III.

Northern blot analysis

Poly(A)$^+$ RNA (1.5 μg) was separated on a 1% agarose gel containing formaldehyde for 3 h at 70 V, using 20 mM MOPS [3-(N-morpholino)-propanesulfonic acid] solution as a running buffer. The gel was blotted to a nylon membrane (Hybond N+, Amersham) [3] with 20× SSC (sodium chloride/sodium citrate) [28]. The denatured RNA was fixed to nylon membranes with UV light (150 mJ), and the blots were prehybridized with 0.5 M sodium phosphate (dibasic and monobasic combination at pH 7.2), 7% SDS (sodium dodecyl sulfate), 1% BSA, 1 mM EDTA and denatured salmon sperm DNA (50 μg/ml) at 65 °C for 4–6 h. After a labeling reaction and denaturation, the purified ^{32}P-labeled cDNA fragments were added to the prehybridization buffer, and the hybridization was carried out at 65 °C overnight. After hybridization, the blots were washed twice for 10 min in 2× SSC, 0.1% (w/v) SDS at room temperature, once in 1× SSC, 0.1% (w/v) SDS for 15 min at 65 °C, and once in 0.1× SSC, 0.1% SDS for 15 min at 65 °C. The blots were exposed to X-ray film (Kodak) for various periods of time depending on the signal intensity. Hybridization signals on developed X-ray film were quantified using a phosphorimager (Fuji-2000, Fuji Photo Film Co. and Kohshin Graphic Systems, Tokyo, Japan).

DNA sequence analysis

Plasmid DNA for nucleotide sequencing was prepared using a DNA plasmid purification system (Qiagen, Chalsworth, CA). Partial nucleotide sequences of cDNA inserts were determined by fluorescence detection method using dye-labeled T3 and T7 primers. Cycle sequencing was performed with a Catalyst 800 Molecular Biology LabStation (Perkin Elmer, Foster City, CA). A model 373A sequencer (Perkin Elmer) was used for automated sequence analysis.

Results

cDNA library construction and differential screening

Poly(A)$^+$ RNA (5 μg) was extracted from 10 min physical impeded maize root tips and used for second-strand cDNA synthesis with a commercial kit based on the Gubler and Hoffman [15] procedure. The cDNAs were size separated by passing samples through a prepared spin column (Sephacryl S-400) and 60 μl frac-

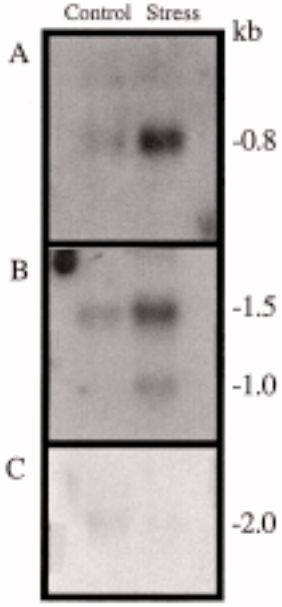

Figure 1. Northern analysis of the expression of transcripts (*pIIG1* and *pIIG2*) in 1 cm maize root tips with (Stress) and without (Control) 10 min physical impedance. 1.5 μg each of mRNA was loaded for each sample. The blot was hybridized with different probes. A. *IIG1* cDNA insert was used as a probe. B. *IIG2* cDNA insert was used as a probe. C. The constitutively expressed actin gene was used to verify that loading of the control lane equaled or exceeded loading of the stress lane.

tions were collected and separated on a 8% agarose gel. cDNAs ranging in size from 0.5 to 1.5 kb, containing *Eco*RI and *Xho*I restriction sites on both ends, were selected as the source material for cDNA library construction. The size selection eliminated smaller cDNA molecules; the larger cDNAs used were more likely to contain complete or nearly complete messages. The primary library contained 5×10^5 original λ phages and was estimated to have contained 97% of the recombinants. 45,000 plaques were differentially screened in duplicate with labeled RNA from control and impeded root tips. Two unique cDNA clones, designated *pIIG1* (impedance-induced gene) and *pIIG2*, which appeared to be regulated by impedance, were chosen for further characterization.

To verify the initial differential expression patterns and determine the transcript size, cDNA clones $pIIG1$ and $pIIG2$ were hybridized to northern blots containing poly(A)$^+$ RNA from control and physically impeded maize root tips.

Identification and cloning of cDNA clones corresponding to poly(A)$^+$ RNAs that accumulate in 10 min physical impedance-treated maize roots

cDNA clone *pIIG1*, whose complementary poly(A)$^+$ RNA was induced in maize root tips by 10 min physical impedance, had a total length of 678 bp. The northern analysis using cDNA *pIIG1* as a probe to hybridize with both control (left lane) and stressed (right lane) poly(A)$^+$ RNAs (1.5 μg each) is shown in Figure 1A. The hybridization signal was strongly induced in impeded (stress) treatments at a poly(A)$^+$ RNA transcript size of about 0.8 kb. Calculation of the signal intensity measured by Phosphorimager revealed a 4.38-fold increase in intensity of the impeded over the control sample. Further verification of the increase in the level of clone *pIIG1* by impedance was achieved by *in situ* analysis (see below).

cDNA *pIIG2* insert consisted of ca. 830 bp. Northern analysis with the *pIIG2* cDNA showed two hybridization bands on the mRNA blot from the stressed treatment shown in Figure 1B. The more strongly expressed band was ca. 1.5 kb in size, while the weaker band was ca. 1.0 kb. The 1.5 kb hybridization band was expressed more strongly in the stress treatment, while the 1.0 kb band was unique to stressed roots. Equal loading of mRNA was verified by reprobing the blots with the actin gene from soybean as shown in Figure 1C [28]. Over expression of the poly(A)$^+$ RNA complementary to *pIIG2* in response to impedance occurred in five additional experiments in which the 1.5 kb band was always increased over 3-fold (phosphoimager data) and the 1.0 kb band consistently appeared in the impeded roots and not in the control roots.

The nucleotide and predicted amino acid sequence of IIG1

Excluding 95 bp of the 5′-untranslated region and 193 bp of the 3′-untranslated region, *pIIG1* contains a 390 bp open reading frame encoding a polypeptide of 129 amino acids with a predicted molecular mass of 13.6 kDa and estimated Pi (isoelectric point) of 6.52 (Figure 2). The deduced amino acid sequence is rich in Leu (17%) and Pro (11%) (Figure 2). It contains a putative hydrophobic signal sequence at the amino terminus because the hydropathy plot showed the presence of very hydrophobic residues at positions 1 through 21 of the deduced amino sequence (Figure 3A). The nucleotide sequence of *pIIG1* had an extremely high similarity (97%) with a previously reported cDNA clone (*pZRP3*), which corresponds to

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1                                          33/~
GG CAC GAG GAG CAC GCA GCG CAG CAC TGA AGC ACC ACT TCC TGC ACT CAC TGT GCT AGC TGC

63/~                                       93/~
TCA AAC CGT AGT AGT AAC AGT CCA TCA CCA CCA ATG GCT CCC AAG GTT GCG CTC TTC CTT
                                               M   A   P   K   V   A   L   F   L
123/10                                     153/20
GCC CTG AGC CTC CTG TTT GGT GCC ACC GCG CAT GGC TGC GAA CCC AAC TGT TCC GGC CCA
 A   L   S   L   L   F   G   A   T   A   H   G   C   E   P   N   C   S   G   P
183/30                                     213/40
GTC GTC CCA ACG CCG CCA GTC GTG CCG ACT CCG TCG TCG CAC AGC CAC GGG CGC TGC CCG
 V   V   P   T   P   P   V   V   P   T   P   S   S   H   S   H   G   R   C   P
243/50                                     273/60
ATC GAC GCG CTC AAG CTC AAG GTG TGC GCC AAC GTG CTA GGC CTC GTC AAG GTC GGC CTA
 I   D   A   L   K   L   K   V   C   A   N   V   L   G   L   V   K   V   G   L
303/70                                     333/80
CCC CAG TAC GAG CAA TGC TGC CCG TTG CTG GAG GGT CTG GTG GAC CTC GAC GCC GCA TTG
 P   Q   Y   E   Q   C   C   P   L   L   E   G   L   V   D   L   D   A   A   L
363/90                                     393/100
TGC CTC TGC ACC GCC ATC AAG GCC AAC GTC CTC GGC ATC CAC CTC CAC GTG CCC CTT AGC
 C   L   C   T   A   I   K   A   N   V   L   G   I   H   L   H   V   P   L   S
423/110                                    453/120
CTC AAC CTC ATC CTC AAC AAC TGC GGC AGG ATT TGC CCA GAG GAC TTC ACT TGC CCC AAC
 L   N   L   I   L   N   N   C   G   R   I   C   P   E   D   F   T   C   P   N
483/130                                    513/~
TAA GCT TGG GAT CCC TTG TGT GCT TCA TCT CGC GAT TCT AAT TAC GAG CAT GTC AAC CTC
 *
543/~                                      573/~
TTG CAA TAT TAG CGA ATA AGT TTG TCG TTT CAA ATT CTT TCG CTG TAC CAT CGG CGA TTG

603/~                                      633/~
TAT TTG TGT GGA GTT ATA TTT GAA ATT TTA ATG ATC AAT AAG AAA TAA TCG TTT TTT AAA

663/221
AAA AAA AAA AAA AAA
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Figure 2. The nucleotide sequence and deduced amino acid sequence of the cDNA insert *pIIG1*. The whole sequence including the poly(A) end is 678 bp. Below the line of the DNA sequence, the longest open reading frame and deduced polypeptide sequence containing a total of 129 amino acids is shown with methionine (M) as a start codon and an asterisk as a stop codon.

an mRNA that accumulates specifically in maize roots and delineates a subset of developing cortical cells [24]. The major difference between *pIIG1* and *pZRP3* was that *pIIG1* had an additional 75 bp in the 5′-untranslated region (Figure 4). A comparison of the deduced polypeptides shows a difference of only 4 amino acids (Figure 4). The differences, although few, were confirmed by sequencing both DNA strands of *pIIG1*. In addition to the homology of clone *pIIG1* with *pZRP3*, a computer-assisted search also revealed significant homology of the deduced polypeptide of *pIIG1* with other proteins including: *pEARLI1* induced by 2 h aluminum exposure in *Arabidopsis* [32]; the cold-induced *CorC* in alfalfa [9], the carrot cDNA *DC 2.15* induced in somatic embryogenesis [4] and the tumor cell-induced *Tid 23* in tobacco [15]. These amino acid sequences were compared to the deduced amino acid sequence of *pIIG1* as shown in Figure 5.

The nucleotide and predicted amino acid sequence of IIG2

Sequencing *pIIG2* from both ends with T3 and T7 primers confirmed a size of 830 pb. Detailed analysis of this sequence suggested an apparent open reading frame of 630 bp which would encode a polypeptide of 210 amino acids with a predicted molecular mass of 23 kDa and Pi 11.98, if translated (Figure 6). This suggested that a 5′-untranslated sequence of ca. 70 bp, and a 3′ non-coding region of about 130 bp were present, which included the poly(A) tail. A search of GenBank (release November 1996) did not reveal any published sequences with significant similarity to *pIIG2*, but a bipartite nuclear targeting sequence was found in the deduced amino acid sequence at position 108–124 (RRQSRRRNERRAGRRRA) [11, 16, 17]). The presence of this sequence suggests that the translated protein would be imported into the nucleus. The deduced amino acid sequence of this gene is shown in Figure 6, and the associated compositional analysis suggests that the protein contains a high percentage of Arg (15%), Val (10%), Ala (10%) and Ser (10%)

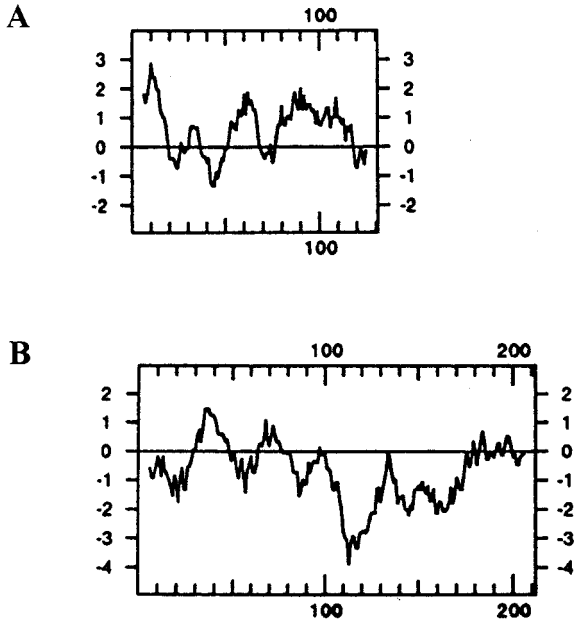

Figure 3. Hydropathy plots of deduced polypeptide sequence of cDNA *pIIG1* (A, top) and *pIIG2* (B, bottom). Hydrophobicity is indicated by positive values and hydrophilicity is indicated by negative values. Plots were drawn by the DNA strider software program and scaled by the Kyte-Doolittle method.

and a very hydrophilic region (Figure 3B). No amino acid sequence repeats were found in this polypeptide. A computer-assisted protein homology search failed to find any known proteins which had significant whole polypeptide sequence similarity to the predicted polypeptide of *pIIG2*.

IIG1 accumulates in protocambium and root cortical cells in response to physical impedance stress

The spatial distribution of *pIIG1* expression in maize subjected to physical impedance was visualized with *in situ* hybridization experiments. Sense and antisense probes consisting of *pIIG1* were hybridized to longitudinal sections of roots of maize seedling which had been subjected to treatments of 0 min and 10 min physical impedance. The results of these analyses are shown in Figure 7. In the absence of physical impedance, the antisense *pIIG1* transcripts were detected only in cortical cell region of the meristem about 0.5 mm to 2 mm behind the root tips and not in the apical meristem or protocambium (Figure 7A). The intensity of the signal was low. However, after 10 min physical impedance, the intensity of *pIIG1* signal increased greatly, not only in cortical cells, but also in the protocambium region (Figure 7B). This result confirms the increased abundance of the mRNA complimentary to *pIIG1* in response to physical impedance seen in northern blots (Figure 1A) and verified by Phosphorimager. Control experiments using sense strand probes showed very low levels of background hybridization and alkaline phosphatase activity (Figure 7C).

Discussion

Because roots constantly experience some degree of physical impedance as they grow through soil, it seems plausable that their response to impedance could be different from the response of aerial organs to wounding, rubbing, shaking and other types of contacts which produce morphogenic changes in the aerial organs. The study reported here was not designed to distinguish such differences, if they exist, but rather to characterize the response to physical impedance of growth applied in a way to minimize any type of thigmomorphic or seismomorphic signal. At an early stage of this project, cDNA probes for three of the touch genes of *Arabidopsis thaliana* [7, 31], kindly supplied by Janet Braam, were used to determine whether physical impedance altered expression of homologous genes in maize. Of the three genes (TCH1, TCH2, TCH3), only TCH1 strongly hybridized with maize poly(A)$^+$ RNA in a northern analysis [22]. TCH1 was strongly expressed at 0, 10, 30 and 60 min of physical impedance with no difference from control expression. In addition, Braam has reported that expression of TCH genes is not promoted by ethylene [7] nor is ethylene production altered in the time that touch gene expression changes, which suggests that they occur in a transduction chain before ethylene or separate from one involving ethylene. After initial screening using these and other known probes, the present experiments were conducted to specifically seek genes regulated in roots by physical impedance.

With differential screening, we found gene expression in maize root tips to be altered after only 10 min of exposure to physical impedance, and two cDNA clones were isolated from a cDNA library. The amount of the mRNA complementary to cDNA *pIIG1* was strongly increased by physical impedance. This cDNA is very similar to the cDNA clone *pZRP3* which encodes a cortical cell-delineating protein [24]. The existence of a multi-gene family of *ZRP3* was confirmed by Southern analysis. Use of *pIIG1* as a hybridization probe with maize genomic DNA digested with either

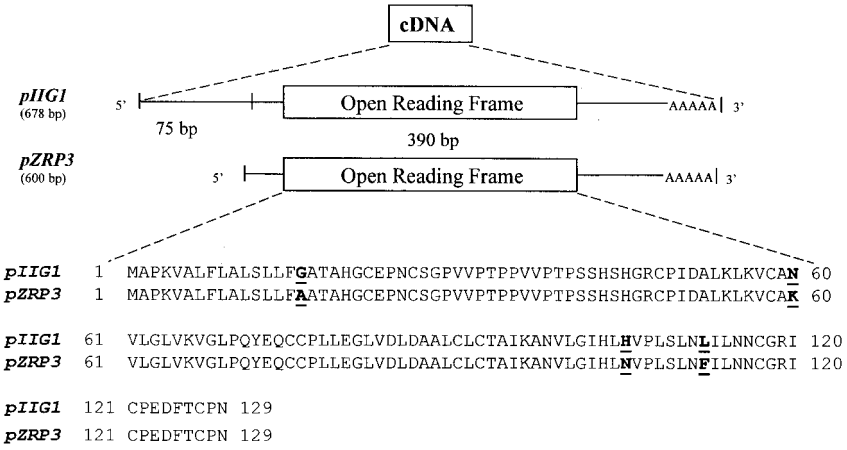

Figure 4. The comparison of the deduced amino acid sequences between *pIIG1* and *pZRP3*. Underlined amino acids indicate the difference between these two sequences. Comparison of the DNA sequences showed an additional 75 bp in 5′-untranslated region of *pIIG1* and sequence similarity of 97% in the opening reading frame and 3′-untranslated region.

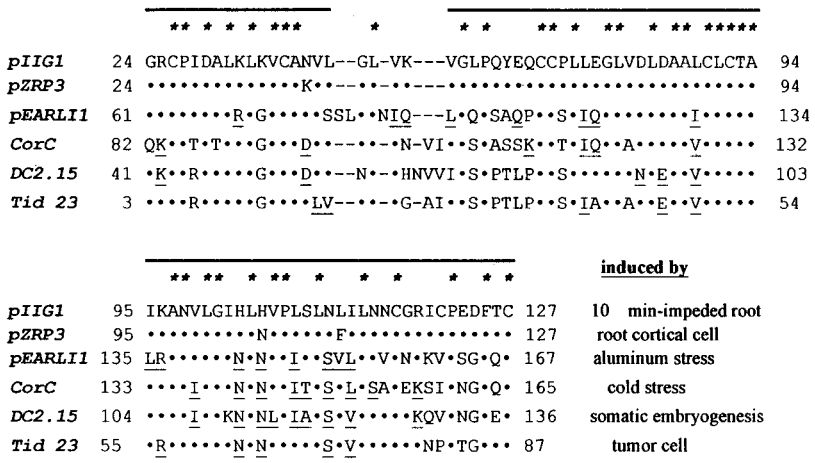

Figure 5. The similarity of the deduced acid sequences between *pIIG1* and a series of proteins. Dots indicate positions of identical amino acids between *pIIG1* and the other polypeptides. Underlined amino acids indicate conserved replacements of amino acids. Dash means gaps that were introduced in the sequence to optimize the alignments and * indicates positions where amino acids are identical in all proteins.

*Bgl*II or *Hin*dIII also revealed several bands (related genes) (data not shown). Thus, both *pIIG1* and *pZRP3* may encode different genes in the same multi-gene family. A GenBank search of the deduced polypeptide sequence also found a significant similarity to two other groups of proteins. One group consists of stress-induced proteins: the *CorC* gene product, a cold-induced protein from alfalfa (*Medicago sativa*) [9] and the *pEARLI1* gene product [32], a protein induced in *Arabidopsis* by 2 h exposure to aluminum stress. The other group consists of developmentally regulated proteins: the protein encoded by carrot cDNA *DC 2.15* induced in the early stage of somatic embryogenesis [4] and a tobacco tumor-related protein encoded by *Tid23* [15]. Even though the functions of these proteins are not known, a cold pretreatment promoted somatic embryogenesis in carrot cell suspensions [26], which indicates that there are relationships between genes responsible for *in vitro* regenerability and stress responses. The similarity between *CorC*, *pEARLI1* and *pIIG1* may indicate that they play similar roles in different stress conditions. John et al. [23] also showed that the cortical cell delineating protein encoded by *ZRP3* accumulated in the inner region of the cortical ground meristem about 0.5 mm to 2 mm behind the root tip in the region of maximum cell division. That finding is similar to our results (Figure 5A) which showed low expression of *pIIG1* in

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1/~                                                 33/~
GG CAC GAG CTG TGG TGC GGG GAG GTC ACG GTG GAG AGC CTC CGT TGG GGG AAT GAG GTC
CTC

63/~                                                93/8
AAG AGG AGG ATG CAG CCC GGC GAC GCC AAC GCC GAG GTC AGC CCC GAG ATG CTC AGG CGA
           M   Q   P   G   D   A   N   A   E   V   S   P   E   M   L   R   R
123/18                                             153/28
ATC AAA AGG GCT AAG AGG GTG AGC CAA ATA TCT GAG AAA GTG GCG ACT GGG ATT TTG TCC
 I   K   R   A   K   R   V   S   Q   I   S   E   K   V   A   T   G   I   L   S
183/38                                             213/48
GGA GTG GTG AAG GTC ACT GGT TAC TTC ACA AGC TCT CTG GCC AAC TCG AAA GCT GGC AAG
 G   V   V   K   V   T   G   Y   F   T   S   S   L   A   N   S   K   A   G   K
243/58                                             273/68
AAG TTC TTC CAA CAT GTT GCC TGG AGA GGA TCC GTT CTT GCT TCG CTT GAC GGA TTT GGG
 K   F   F   Q   H   V   A   W   R   G   S   V   L   A   S   L   D   G   F   G
303/78                                           333/88
GAA GAT CTT GCG ACG CCC GTG GAG GGT GGC CGG AAA GAA CGT TTT GTC CAC GTC GTC AAC
 E   D   L   A   T   P   V   E   G   G   R   K   E   R   F   V   H   V   V   N
363/98                                             393/108
TGT GAC GAC CGG GCT AGT ATC TCA CAG GTA CGG AGA CAA AGC CGC CGC CGC AAC GAA CGA
 C   D   D   R   A   S   I   S   Q   V   R   R   Q   S   R   R   R   N   E   R
                                            Bipartite nuclear targeting sequence
423/118                                            453/128
AGG GCT GGA CGC CGC CGG GCA CGC CAT CGG GAC GGC GTG GGC CGT GTT CAA GAT CCG GCA
 R   A   G   R   R   R   A   R   H   R   D   G   V   G   R   V   Q   D   P   A
483/138                                            513/148
GGC CTT GAA CCC CAA GAG CGT CCT CAA ACC CAC GGC GCT GGC CAC GTC CAC CAT CAG GGC
 G   L   E   P   Q   E   R   P   Q   T   H   G   A   G   H   V   H   H   Q   G
543/158                                            573/168
CAA CGT TGC CGA GCT TCG CGC GAT GCA CGG CAG CAG CAA GTA GCT CGC GCC TGC CGT CCC
 Q   R   C   R   A   S   R   D   A   R   Q   Q   Q   V   A   R   A   C   R   P
603/178                                            633/188
CGT TTC GTA AAC TCT CTA TTA TCT CGC TCT GTC ACG ACC AAC GAT GCA CTC GCT GCT TCC
 R   F   V   N   S   L   L   S   R   S   V   T   T   N   D   A   L   A   A   S
663/198                                            693/208
AGC AGC AGC GTT GGC CGT TGG CCT GTA AAT TCG TGT GGC TGA AAC TGG GAA AGC CAG GAA
 S   S   S   V   G   R   W   P   V   N   S   C   G   *
723/~                                              753/~
CTG AAA GGC TTA CCG CTT CCG CTT TGT TAC TTT GTT AGT GAT GCT GGT GAT GTT CTA AGA

783/261                                            813/271
GCT TTT ACC ACT GCT GTA AAA AAA AAA AAA AAA AAA AAA AAA AA
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Figure 6. The nucleotide sequence and deduced amino acid sequence of the cDNA insert *pIIG2*. The whole sequence including poly(A) end is 826 bp. Below the line of the DNA sequence, the longest open reading frame and the deduced polypeptide sequence containing a total of 210 amino acids is shown with methionine (M) as a start codon and an asterisk as a stop codon.

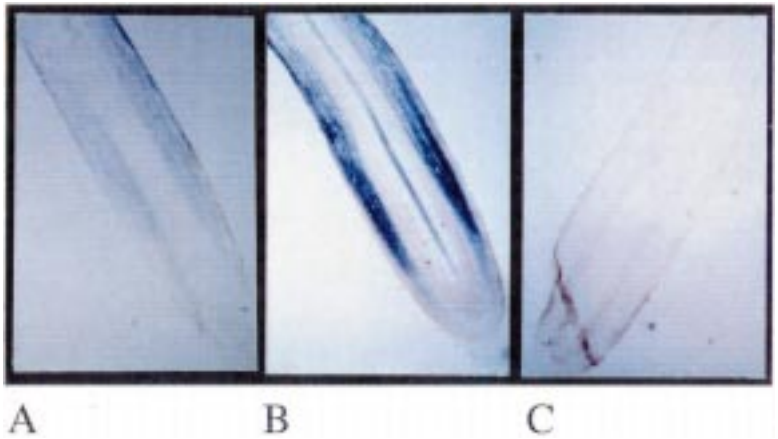

Figure 7. In situ localization of *IIG1* mRNA in maize root tips. A. Maize root in triaxial cell hybridized with the *IIG1* antisense probe. B. Maize root in triaxial cell with 10 min physical impedance hybridized with the *IIG1* antisense probe. C. Maize root in triaxial cell hybridized with the *IIG1* sense probe.

cortical cells of roots of unimpeded seedlings. However, physical impedance induced *pIIG1* expression in both protocambial and cortical cells (Figure 5B). Physical impedance induces ethylene biosynthesis in one hour [20, 33], but in fact, the induction of *pIIG1* occurs much earlier than the earliest observed effect on ethylene production. The evidence suggests that the gene encoding *pIIG1* may be activated earlier in the response to physical impedance than is ethylene production.

cDNA *pIIG2* was 830 bp in length, and its deduced sequence encoded a hydrophilic polypeptide of 210 amino acids (Figure 6). Northern analysis yielded two hybridization bands (1.5 kb and 1.0 kb) (Figure 1B). Use of *pIIG2* as a hybridization probe with maize genomic DNA digested with *BamHI*, *Bgl*II, *Hin*dIII and *Kpn*I revealed one strong band in each lane and up to four less distinct bands in other lanes (data not shown). The same probe hybridized to reveal one strong band in rice DNA not digested and that digested with *Xba*I. These Southern analysis results led us to believe that *pIIG2* is part of a small multigene family and that the two bands seen in Figure 1 represent two members of that family. At this time we do not have evidence to disprove other possible explanations for the occurrence of two bands in the northern analysis of *pIIG2*.

A GenBank search did not reveal homology of *pIIG2* with any published sequence. The occurrence of a bipartite nuclear targeting sequence suggests that the gene product may function in the nucleus. A portion of the deduced polypeptide structure (residues 100–173) contained a high percentage of arginine. Arginine is a basic, positive charged amino acid which can neutralize and bind DNA sequences in the nucleus. The high arginine content region suggests that the *pIIG2*-encoded protein may function in binding to nuclear chromosomes and thus represent a DNA-binding protein or transcription factor. It seems reasonable that in the initial stages of the response to physical impedance, before transcription of most functional genes is altered, some transcription factors and trans-acting proteins which can control and activate the expression of more specific genes would be required and should be expressed. However, transcription factors are often very low abundance proteins and detection of a message for a low abundance protein is inconsistent with the strong expression of *pIIG2* detected by northern analysis (Figure 1B). Thus the protein encoded by *pIIG2* may have some other function. Regardless of the function, the timing of the increase of *pIIG2* transcripts indicates that this event occurs early in the response of roots to physical impedance.

The primary finding of this study is that the abundance of the transcript which encodes *pIIG1* increased several-fold after only 10 min physical impedance. That timing, which is earlier than the earliest evidence for increased ethylene production, plus the fact that similar genes are stress and developmentally regulated in other systems, suggest that the gene which encodes cDNA *pIIG1* may be a component in the early steps of the signal transduction of stresses, and that its regulation preceeds the regulation of ethylene biosynthesis. Our data also suggest that early events in the signal transduction pathway may involve proteins, like the peptide encoded by *IIG2*, which act in the nucleus to mediate responses to physical impedance.

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Note added in proof

Two *Arabidopsis thaliana* sequences with high, same frame homology to *pIIG2* have been deposited in GenBank under accession numbers AC003952 (24 Dec. 1997) and AL022373 (09 April 1998).

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