Fast changes in expression of expansin gene and leaf extensibility in osmotically stressed maize plants

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Abstract

Adding PEG to the nutrient medium of maize (Zea mays L., hybrid Harkovskaya 310 MV) plants arrested the growth of their leaves initially but in 40–50 min growth resumed. This coincided with and was obviously due to a gradual increase in extensibility of the primary leaf suggested by changes in its extension rate, which was induced by adding a counterweight to inductive electromechanical position sensor. Specificity of gene probe for expansins was confirmed by sequencing cDNA and its comparison with literature data. Dot-blot analysis showed an increase in transcript level of expansin genes induced by PEG treatment. Thus gene-specific regulation of expansin mRNA pools likely contributes to fast adjustment of cell wall-loosening under conditions of water deficit.

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1. Introduction

Plants often experience reduced availability of water caused by an increase in evaporation demand due to wind, fluctuations in illumination intensity and in temperature. Transient increase in water deficit in plants might also be due to uneven distribution of moisture in soil and penetration of rapidly growing roots into dry soil patches, which inevitably creates sudden problems for water uptake. One predictable consequence of increased leaf water deficits is an inhibition of leaf elongation. Since cell extension is driven by turgor generated by water uptake it is not surprising that many experiments have shown immediate cessation of growth when leaf water potential is suddenly reduced by increased evaporational demand and/or by reduced availability of water from roots [9,14,18]. However, cessation of growth under these conditions is sometimes transient and a partial or complete recovery is observed even though the environmental conditions imposing the stress remain unchanged (see above cited references). This ability allows leaf area expansion to resume thus enhancing photosynthetic potential. It is important to understand how the recovery in leaf elongation is achieved. A likely contributing factor is cell wall extensibility [8,16]. McQueen-Mason et al. [12] identified a class of wall-associated proteins called expansins that can directly modify the mechanical properties of plants cell walls leading to turgor-driven cell expansion. Genes coding for these proteins were identified in many plant species and their expression followed in different parts of maize plants [20]. Some results show strong correlation between expansin gene expression and growth rate although this correlation is not absolute since elongation growth is likely to be controlled by expansin acting in concert with other factors [2]. Changes in expansin transcript level were shown to correlate with the maintenance of root growth and increase in wall extensibility at low water potentials [21]. The aim of the present research was to find out if modification of expansin transcript level might be responsible for the control of leaf growth under conditions of water stress created by adding PEG to nutrient solution.
2. Results

Fig. 1 shows the sharp decline in growth rate of leaves shortly after addition of PEG to the nutrient solution. Growth reduction was followed by a transitory leaf shrinkage and then no growth was observed during next 50 min. Partial recovery of growth occurred to give a new stable growth rate about 50% of the initial values. A counterweight added during the period of PEG-induced cessation of growth caused leaf extension, the rate of which changed with time after the start of treatment. Several additions of the single weight (2 g) to the position sensor during the period of absence of growth induced extension with ever growing rate (Fig. 2 and Table 1).

Comparison of sequence of cloned region of DNA with the published data [21] showed its identity to a \textit{EXPA} gene.

The results of dot-blot analysis of mRNA transcripts for expansin gene using $^{32}$P-labeled DNA probe (Figs. 3 and 4) showed their accumulation following PEG treatment. The increases in expansin gene expression reached its maximum by 30 min after addition of PEG to the nutrient medium followed by a subsequent decline in transcription activity although remaining higher than in control.

3. Discussion

The inhibitory effects of PEG on whole plant growth can exceed the effects of other osmolytes such as NaCl, which has been ascribed to reduced oxygen availability and inhibition of water flow in PEG solutions [3]. However both NaCl and PEG treatment resulted in similar growth responses in our experiments (data not shown). Cessation of the growth of maize leaf after addition of PEG to the nutrient medium has been attributed to cell wall hardening [4]. However under conditions used in our experiments leaf shrinkage was observed suggesting another explanation of PEG-induced immediate growth response. Leaf shrinkage is known to result from a decrease in leaf turgor [13], which in our case might be due to a decline in water delivery from the roots. While the immediate decline of the leaf growth rate results from a reduced water supply itself, growth resumption observed later demands some other explanations.

Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Time of PEG action (min)</th>
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<tbody>
<tr>
<td>((X_1 - X_0)) ((\mu\text{m min}^{-1}))</td>
<td>10</td>
</tr>
<tr>
<td>7.7 ± 1.4</td>
<td>12.3 ± 1.2</td>
</tr>
</tbody>
</table>
Previous observations that wall properties may change quickly in response to changes in growing conditions make it possible that prompt restoration of leaf growth in stressed plants may be, in some measure, a consequence of increases in extensibility and possibly decreases in the cell wall yield threshold [16–18]. This would be detectable as an increase in leaf extensibility. To check this possibility, an additional 2 g weight was supplied to the balance of the position sensor of the electromechanical position sensor used to monitor leaf elongation. Several additions of the same counterweight during the period of PEG-induced arrest of growth caused leaf extension, the rate of which increased with time after the start of treatment, evidencing an increase in leaf extensibility. This resembles the results of experiments, where an increasing series of weight was applied, which induced ever increasing extension rate [6]. Since in our case the same weight (2 g) was used, the observed increase in extension rate suggests an increase in cell wall extensibility. Extension response is known to depend on water flow [5]. However since PEG-induced water stress was shown to inhibit hydraulic conductance [11], the increase in leaf extension rate observed in our experiments is not likely to be due to changes in availability of water necessary for maintaining extension. Single force and not a series of forces were applied in our experiments, which did not allow us to distinguish if wall yielding coefficient or yield threshold were changed [5]. Nevertheless changes in leaf extension rate observed in our experiments are likely to be due to some modifications in cell wall extensibility. Thus partial restoration of growth was preceded by and might be due to the increase in cell wall extensibility.

PEG-induced modification of expansin transcript level was studied in attempt to reveal possible involvement of expansins in the control of changes in cell wall extensibility. We focused on the sequence of alpha-expansin (EXPA1) gene known to be expressed in maize leaves [20]. It shows accumulation of expansin transcripts preceding an increase in leaf extensibility and growth resumption following PEG treatment. Thus the results are consistent with the hypothesis that the adaptive wall-loosening and growth maintenance in leaves in response to low water potential of nutrient medium are the results at least in part of altered expansin gene expression in the leaves.

4. Methods

4.1. Plant material

Seedlings of maize (Zea mays L., hybrid Harkovskaya 310 MV) were grown in darkness at 25 °C between glass tubes sealed at the ends, tired together and floated on 0.1 strength Hoagland–Arnon nutrient solution. Three days later the seedlings were grown on under 400 m–2 s–1 PAR from ZN and DNAT-400 fluorescent lamps, at a 14-h photo-period and 25 °C. The plants were used when 1-week-old. Polyethylene glycol (PEG 6000) was added to the bubbled nutrient medium to produce a concentration of 15% (wt/wt), which lows its osmotic potential to ~0.5 MPa.

4.2. Measurement of growth rate

Extension rate of the primary leaf was measured by means of an analogue inductive electromechanical position sensor [10]. Output signals from the sensor were tracked continuously using a chart recorder. To estimate leaf extensibility an additional weight (2 g) was added and withdrawn every 10 min during the period of absence of growth arrested by PEG treatment. Elongation rate was calculated from the slope of the linear regression between 3 and 10 min after each weight addition.

4.3. Expression analysis

Transcriptional activity of expansin genes was estimated by means of dot-blot-analysis. For this purpose RNA was isolated with guanidine thiocyanate buffer [1] from samples of growing part of the primary leaves prior to and after addition of PEG to the nutrient medium (the growth zone of the primary maize leaves of this age was established in preliminary experiments, in which ink pots were put on leaf surface and growth of different parts of the leaves was followed). Leaves of five plants were pooled into one sample. After deproteinization with the mixture of phenol and chloroform RNA was precipitated with isopropanol. RNA pellet was dissolved in autoclaved water with 0.05% diethylpyrocarbonate added and reprecipitated with 6 M LiCl. Concentration of total RNA was determined on spectrophotometer SmartSpec™ Plus (Bio-Rad). Dot-blot analysis was carried out as described [15] in hybridization chamber Micro-4 (Hybaid) at 65 °C.

The fragment of the 3’ UTR of the alpha-expansin 1 gene was used as a gene-specific probe [20]. It was PCR-amplified with primers Ex1. For 5’-CTACTCTACTCCATCGACG-3’ and Ex1.Rev 5’-ATTAAGTTGCACGACACC-3’ [20]. Ob-
tained product of amplification 259 bp in size was cloned in vector pGEM-T Easy. Sequence determination of the insert was carried out in ABI PRISM 310 Genetic Analyzer with BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit. Plasmid DNA of cloned region of expansin gene isolated by the method of mild lysis was used for the probe preparation. Synthesis of uniformly labeled DNA probes was carried out using primers mentioned above in the presence of $[^{32}P]-dNTP$ [7]. rRNA genes serve as a reference in dot-blot analysis. Hybridization of filters containing bound RNA with radiolabeled probe they were dried and exposed on X-ray film. After hybridization of filters containing bound RNA with radiolabeled probe they were dried and exposed on X-ray film. Densitometry measurements of hybridization spots are expressed as per cent of data obtained for plants untreated with PEG and sampled prior to and over the course of experiment. Experiments were repeated three times with three chemical replicates each.

Acknowledgements

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References