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Research article

Expression of asparagine synthetase genes in sunflower (*Helianthus annuus*) under various environmental stresses

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Abstract

In sunflower, asparagine synthetase (AS; EC 6.3.5.4) is encoded by a small family of three genes (*HAS1*, *HAS1.1* and *HAS2*) that are differentially regulated by light, carbon and nitrogen availability. In this study, the response of each gene to various stress conditions was examined by Northern analysis with gene-specific probes in leaves and roots. The expression of *HAS1* and *HAS1.1* genes was induced by osmotic stress (300 mM mannitol), salt stress (150 mM NaCl), and heavy-metal stress (20 μM $CuSO_4$), more in roots than in leaves. The expression of *HAS2* was not significantly altered by stress treatments. The positive response of *HAS1* and *HAS1.1* genes to osmotic and salt stresses occurred in the light, in contrast to that previously found in unstressed plants. Measurements of sucrose and total free amino acid contents in leaves and roots indicate that the expression of root *HAS1* and *HAS1.1* genes in stressed plants is not under metabolic control by the intracellular C/N ratio, suggesting the involvement of some specific stress factor(s). Growth of plants at 40 °C for 12 h negatively affected the expression of *HAS1* and *HAS1.1* but not that of *HAS2*.

Keywords: Abiotic stress; Asparagine synthetase; Gene expression; *Helianthus annuus*; Nitrogen metabolism

1. Introduction

Unfavourable growth conditions, such as high temperature, exposure to heavy metals, soil salinity, or nutrient deficiency [5,6,11,12], can reduce the photosynthetic capacity of the plant, resulting in a decreased carbon availability and an enhanced protein degradation which lead to internal ammonium accumulation [11]. The synthesis of amides, especially asparagine, is stimulated by some environmental stresses [1,16,22]. Post-harvest stress increased the content of asparagine in asparagus spear tips [13]. In tomato, the asparagine pool also increased markedly under deficiency of zinc or excess of cadmium [1,22]. Accumulation of asparagine in response to abiotic stresses could be an ammonium detoxification mechanism and a way to store nitrogen when protein synthesis is inhibited by stress conditions. Likewise, amide accumulation occurs in plants when high amounts of ammonium are internally generated by deamination of soluble amino acids released from proteolysis during leaf senescence, thus preventing ammonium toxicity [7].

Asparagine is synthesized in plants by asparagine synthetase (AS), which catalyses the transfer of the amide group from glutamine (or ammonium) to aspartate, rendering asparagine and glutamate. AS is encoded by a variable number of genes in each species. Three distinct genes encoding functional AS have been identified in sunflower: two class I

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(*HAS1* and *HAS1.1*) and one class II (*HAS2*) AS genes [8]. Class I and class II AS genes are differentially regulated by light, metabolites, and the internal C/N balance [8,9,14], and therefore they might play different roles in different situations requiring asparagine synthesis for nitrogen mobilization and/or storage. A close relationship between the abundance of AS transcripts and free asparagine levels has been found in *Arabidopsis thaliana* [14] and in sunflower [9], showing that transcription is a relevant step in the regulation of asparagine synthesis.

Neither the extent of participation of distinct AS genes nor the regulation of their expression under stress conditions are well established. In this paper, the expression patterns of *HAS1*, *HAS1.1* and *HAS2* genes in leaves and roots of sunflower under different stress conditions, and their relationships with the C/N status of the plant, were investigated.

2. Results

2.1. Effects of osmotic stress and salt stress on AS gene expression and on sucrose and amino acid contents in sunflower plants

In order to evaluate the response of sunflower AS genes to osmotic stress or salt stress, the standard nutrient solution was supplemented with 300 mM mannitol or 150 mM NaCl, respectively. *HAS1* and *HAS1.1* genes exhibited a very similar expression pattern and clearly different from that of *HAS2* gene (Fig. 1). Mannitol and salinity promoted a remarkable increase of *HAS1* and *HAS1.1* transcripts in root tissue after 24 h of treatment. Induction of both genes by mannitol was already observed as early as 4 h of stress treatment. In leaves, the intensity of induction was much lower than in roots, and only became visible after 24 h. The levels of transcripts in leaves were higher in mannitol-treated than in salt-treated plants. It was reported [9] that light represses the expression of *HAS1* and *HAS1.1*. Results in Fig. 1 confirm the low level of expression of both genes in leaves of illuminated unstressed plants, and show that they are partially induced in the light under osmotic and salt stresses. Expression of *HAS2* was not significantly affected by mannitol or salinity.

Sunflower AS genes are metabolically regulated by the internal C/N balance [9]. To determine whether the higher expression of *HAS1* and *HAS1.1* genes in response to osmotic and salt stresses could be related to changes in the intracellular C/N ratio, the contents of sucrose and total free amino acids were analysed in unstressed and stressed plants (Fig. 2). Root amino acid content was unaffected after 24 h-treatment with mannitol or NaCl. By contrast, the sucrose content decreased in mannitol-treated plants and dramatically increased in saline conditions, compared to control plants. In leaves, the levels of sucrose and free amino acids were both increased by mannitol- and salt-treatments.

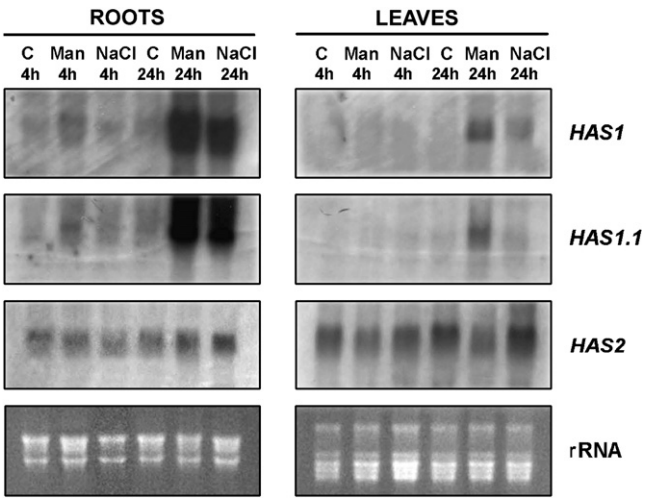

Fig. 1. Effect of osmotic stress and salt stress on the expression of *HAS1*, *HAS1.1* and *HAS2* genes in roots and leaves of sunflower plants. To induce osmotic stress and salt stress, the standard nutrient solution was supplemented with 300 mM mannitol (Man) and 150 mM NaCl, respectively. At the indicated times, roots and leaves from control (C) and stressed plants were sampled. Twenty micrograms of total RNA from the samples were electrophoresed, stained with ethidium bromide (rRNA) and blotted onto a nylon filter. The filter was hybridized sequentially to probes specific for *HAS1*, *HAS1.1* and *HAS2*. The old probe was stripped off the filter before each new hybridization. Results of hybridization was revealed by autoradiography after 15 d of exposure.

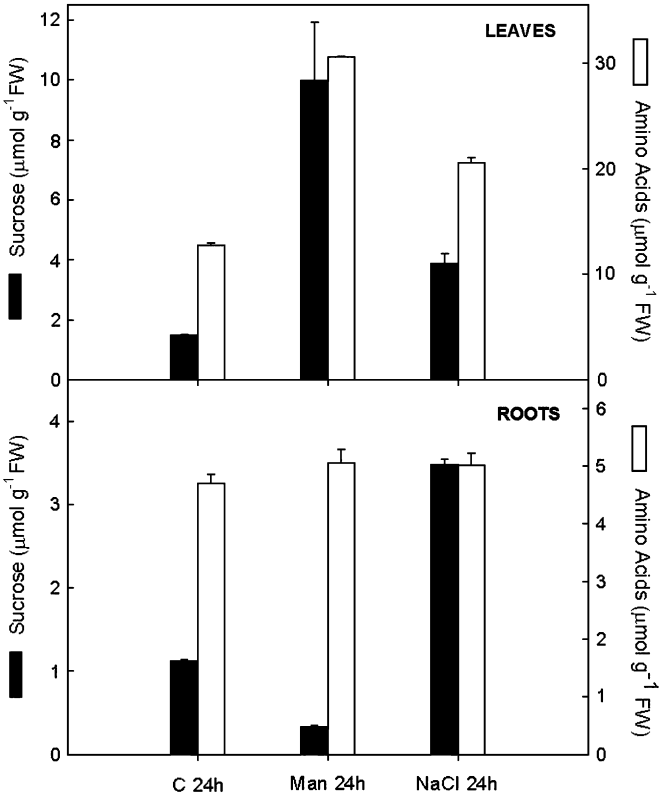

Fig. 2. Sucrose and total free amino acid contents in roots and leaves of sunflower plants under osmotic stress and salt stress. Roots and leaves sampled from the plants used in the experiment depicted in Fig. 1 were analysed for sucrose (full bars) and total free amino acids (empty bars). Each value is the mean ± SD of the results from three separate plants, and measured in duplicate. C: control; Man: mannitol.