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Research article

Low-temperature effect on enzyme activities involved in sucrose–starch partitioning in salt-stressed and salt-acclimated cotyledons of quinoa (*Chenopodium quinoa* Willd.) seedlings

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ABSTRACT

The effect of low temperature on growth, sucrose–starch partitioning and related enzymes in salt-stressed and salt-acclimated cotyledons of quinoa (*Chenopodium quinoa* Willd.) was studied. The growth of cotyledons and growing axes in seedlings grown at 25/20 °C (light/dark) and shifted to 5/5 °C was lower than in those only growing at 25/20 °C (unstressed). However, there were no significant differences between low-temperature control and salt-treated seedlings. The higher activities of sucrose phosphate synthase (SPS, EC 2.4.1.14) and soluble acid invertase (acid INV, EC 3.2.1.25) were observed in salt-stressed cotyledons; however, the highest acid INV activity was observed in unstressed cotyledons. ADP-glucose pyrophosphorylase (ADP-GPPase, EC 2.7.7.27) was higher in unstressed cotyledons than in stressed ones. However, between 0 and 4 days the highest value was observed in salt-stressed cotyledons. The lowest value of ADP-GPPase was observed in salt-acclimated cotyledons. Low temperature also affected sucrose synthase (SuSy, EC 2.4.1.13) activity in salt-treated cotyledons. Sucrose and glucose were higher in salt-stressed cotyledons, but fructose was essentially higher in low-temperature control. Starch was higher in low-temperature control; however, the highest content was observed at 0 day in salt-acclimated cotyledons. Results demonstrated that low temperature induces different responses on sucrose–starch partitioning in salt-stressed and salt-acclimated cotyledons. Data also suggest that in salt-treated cotyledons source–sink relations (SSR) are changed in order to supply soluble sugars and proline for the osmotic adjustment. Relationships between starch formation and SuSy activity are also discussed.

1. Introduction

Seedling establishment is a critical process to plant growth, especially under adverse environmental conditions [7]. Within adverse conditions, soil salinity and low temperature are perhaps the more important stress factors that determine the geographic distribution of plants and their productivity [2]. Seedling injuries caused by these stresses generate a great damage to agriculture reducing the yield and quality of products [7]. During seedling establishment low temperature and salinity modifies several biological processes such as osmotic homeostasis, photosynthetic carbon fixation, carbon partitioning, carbohydrate and lipid metabolisms, and gene expression [7,15]. Seedlings adapt to salinity and low temperature by different mechanisms, including changes in morphological and developmental pattern as well as physiological and biochemical processes [13,16,26,31,34,40]. Adaptation is associated with maintaining osmotic homeostasis by metabolic adjustments that lead to the accumulation of metabolically compatible compounds such as carbohydrates, polyols, betaines and proline [7,44]. These organic osmolytes can accumulate to high concentrations without disturbing intracellular biochemistry [7]. Apart from their role in osmotic adjustment, compatible solutes also have osmoprotective functions. Due to their specific hydrophilic structure, they are capable of replacing water on the surfaces of proteins, protein complexes or membranes, thus preserving their biological functions [7]. Most compatible solutes also seem to play an important role in hydroxyl radical scavenging thus defending seedlings against oxidative damage, which is a common consequence of many abiotic stresses [27]. Sucrose is the more commonly accumulated free sugar in response to low temperature and salinity stresses [13,15,33]. Nevertheless, carbohydrate accumulation is not limited to only sucrose; lesser amounts of glucose, fructose, and another sucrose-derived oligosaccharide family

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Abbreviations: Acid INV, soluble acid invertase; ADP-GPPase, ADP-glucose pyrophosphorylase; AMG, amyloglucosidase; G6PDH, glucose-6-phosphate dehydrogenase; PGM, phosphoglucomutase; PPFD, photosynthetic photon flux density; SPS, sucrose phosphate synthase; SuSy, sucrose synthase.

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have also been observed in salt and cold stressed plants [31,41]. Moreover, low temperature and salinity stresses often involve changes in starch content [14,20,40]. This fact is very important because in many seeds, including mono- and dicotyledonous species, starch is the main carbon reserve [30].

According to several studies seedling adaptation to saline and cold stresses also includes modifications of source–sink relations (SSR) and sucrose–starch partitioning [2,22,33]. Modification of SSR typically shifts the concentration of metabolites, mainly sucrose and starch, in the tissues. Sufficiency soluble sugars supply is known to activate various carbohydrate-consuming cell functions such as biosynthesis of polysaccharides and proteins, cell cycle, respiration, and nitrogen assimilation [17]. Sugars can also act as signal molecules and regulate gene expression of different physiological cycles [10,15,44]. However, despite the clear alterations in growth and photosynthetic metabolism that occur in response to salt and low-temperature stress, the exact nature of the changes in carbon metabolism that are induced by these stresses have not still been completely elucidated [7]. In this context, it has been found that salinity and low temperature induce synthesis of polyols (linear polyhydric sugar alcohols) and cyclitols (cyclic polyhydric sugar alcohols) from the more common storage carbohydrates such as starch and sucrose [13,14]. These observations have led to the suggestion that naturally occurring changes in the carbohydrate status may have an adaptive role in allowing seedlings to survive under saline and/or low-temperature conditions [12,31,38,41]. However, under field conditions stress does not generally come in isolation and many stresses act simultaneously. In response to these stress signals that cross- talk with each other, seedlings have developed diverse pathways for combating and tolerating them [15]. Thus, when different stresses co-occur seedling responses are usually unpredictable by a single factor of analyses, and so they are unique and cannot be directly extrapolated from the response of seedlings to each stress applied individually. In addition, the simultaneous exposure of seedlings to different abiotic stress conditions will result in the co-activation of different stress-response pathways, which can result in intensification, overlapping or reversal of stress effects [13]. Therefore, it is necessary to understand how seedlings respond to combined stress conditions.

Many studies have demonstrated quantitative and qualitative changes in the carbohydrate content of seedlings exposed to low temperature and salinity [33,43,45,51] but the literature available on combined effects of both stresses is scarce [13,38]. Consequently, it still remains unclear whether the positive effects of a particular carbohydrate are interchangeable between saline and low-temperature stress, or whether the carbohydrates fulfil specific roles under these stress conditions when they act simultaneously. The aim of this study was to investigate specificity of changes in SSR of quinoa cotyledons in relation to growth, proline, and sucrose–starch partitioning and related enzymes, as a consequence of simultaneous exposure of seedlings to salinity and low-temperature stresses. Quinoa, the world's potential new crop, was chosen as experimental material because it is a well adapted crop to adverse abiotic factors and exhibits a fast growth during seedling establishment [23]. Moreover, in the cotyledoneous, two- and five-leaf stage, quinoa has a high frost resistance with no damage at low temperatures; however, frost exposure during flower bud formation and anthesis has a serious negative effect [18].

2. Results

2.1. Growth measurements

Fig. 1 shows the growth of cotyledons and growing axes in stressed and unstressed quinoa seedlings. In salt-stressed and salt-acclimated by the fourth day cotyledon growth was already beginning to slow down but from the sixth day on further growth occurred. In unstressed and low-temperature control cotyledons a sustained increased during the experimental period was observed; however, it was higher in the first ones (Fig. 1A). Interestingly, from the sixth day there were no significant differences in the growth of salt-treated and low-temperature control cotyledons. Growing axis length was also higher in unstressed seedlings than in stressed and low-temperature ones, but there were no significant differences between stressed and low- temperature seedlings during the experimental period (Fig. 1B). In unstressed seedlings a delay in growth rate was observed from the ninth day, which was coincident with emergence of the first pair of leaves.

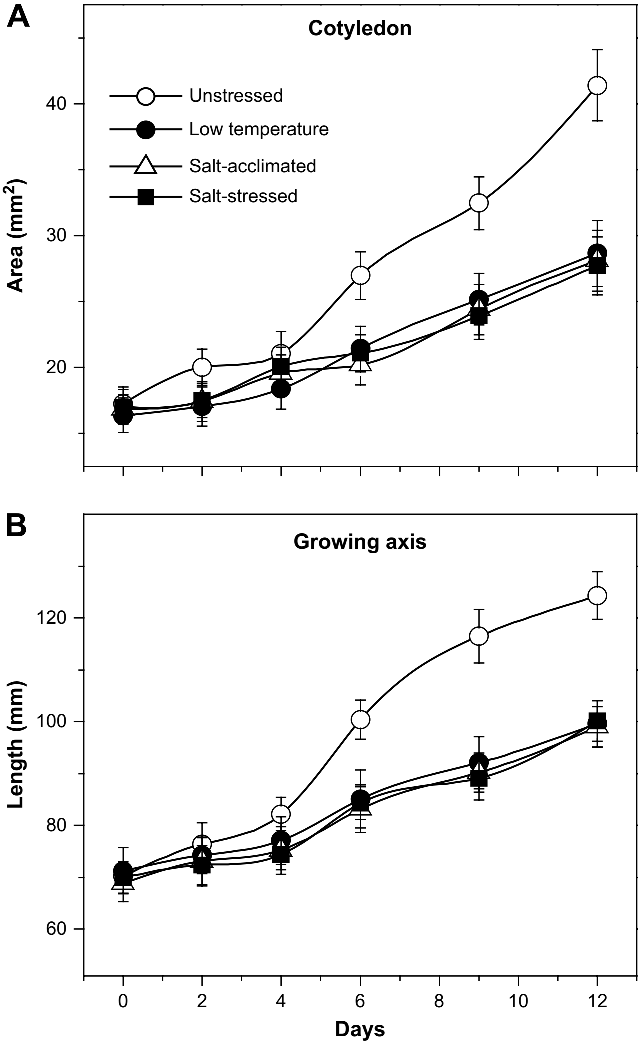

Fig. 1. Changes in cotyledon area (A) and growing axis length (B) during growth and development of quinoa seedlings. Cotyledon area corresponds to one pair. Values are means ± SD of three different experiments ($n = 20$).

2.2. Changes in enzyme activities

In our study, we examined the major regulatory enzymes involved in the carbon flux of seedlings exposed to low temperature and saline stresses (Fig. 2). Measurements of the activity of SPS exposed a low temperature showed different patterns in both salt-stressed and salt-acclimated cotyledons when compared with low-temperature and unstressed control cotyledons (Fig. 2A). The total