

Activity of Sucrose Synthase and Acid Invertase in Wheat Seedlings During a Cold-shock Using Micro Plate Reader Assays

Amani Abdel-Latif

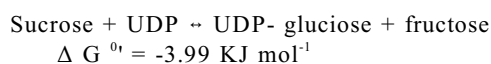
Department of Botany, Faculty of Science, Alexandria University, Egypt.

Abstract: The effect of cold-shock (2 - 4°C) on wheat (*Triticum aestivum*) sucrose metabolism is investigated. Data on changes of apparent activities of sucrose synthase (SS) and acid invertase were determined in small volumes using a novel micro plate reader system which combined high rates of activity with good reproducibility and high sample throughput. Both enzymes exhibited parallel increase after 2, 4 and 6 hours of transferring the seedlings to cold conditions. These results indicate that both enzymes are involved in the plant response to chilling stress.

Keywords: Acid invertase, sucrose synthase, cold acclimation, wheat

INTRODUCTION

Low temperature is a major environmental limitation on plant geographical distribution and productivity. Many tropical and subtropical plants are less tolerant against low temperature and are easily damaged by chilling temperatures (Karlson *et al.*, 2002). In contrast, over wintering plants are capable of exhibiting high levels of cold tolerance, which is acquired through the process of cold acclimation. A sudden decrease in temperature at which plants are growing is known as a chilling stress. Its effect in plant metabolism may be different according to plant species, but it is generally accompanied by an immediate cessation of plant growth while photosynthesis continues at a decreased rate (Levitt, 1980; Tognetti *et al.*, 1989; Vestlov *et al.*, 2002). The result is a net accumulation of photosynthetic products due to the growth stoppage. In many plants this is seen as an accumulation of sucrose and oligosaccharides derived from it (Einig *et al.*, 1991). Plants contain two types of enzymes capable of cleaving sucrose. One is sucrose synthase (UDP-glucose: D-fructose-2-glucosyl transferase, EC 2.4.2.13) which catalyzes the reaction:



The other type of enzymes is invertase (EC 3.2.1.26) which catalyzes the essentially irreversible hydrolysis of sucrose to glucose and fructose:



$$\Delta G^{\circ} = -29.3 \text{ KJ mol}^{-1}$$

Calderon and Pontis (1985) showed that the activity of sucrose synthase (SS), one of the sucrose metabolizing enzymes, starts to increase within 1 hr. after the beginning of the cold stress and continues rising during the acclimation period. In this communication we present data on changes in apparent activities of acid invertase and sucrose synthase during chilling stress. They are based on improved methods for the sensitive assay of these enzymes using a micro plate reader system.

MATERIALS AND METHODS

Growth Conditions:

Wheat seedlings were grown in vermiculite and irrigated with half strength Hoagland solution in a growth chamber at 25°C for a week with a day/night regime of 14/10 hr. Irradiance at the plant canopy was 100 W.m⁻². Seven days following emergence, Seedlings were transferred to 4°C with the same day/night regime and irradiance. Chilling treatments were always started at the beginning of daytime (0700 hr.).

Corresponding Author: Amani Abdel-Latif, Department of Botany, Faculty of Science, Alexandria University, Cairo.
Email: amani@uqu.edu.sa.

Enzyme Extraction and Assay:

Sucrose Synthase:

Wheat leaves were excised 2, 4 and 6 hours after imposing the chilling treatment. One gram of leaf tissue was homogenized with liquid nitrogen using a "Microdismembrator "(Braun, Melsungen, FRG). The leaf homogenate (4mg; particle size below 100 µm) was mixed with 100 mg polyclar AT and extracted for 5 min. with 1 ml of ice-cold Tris/Borate/β-mercaptoethanol buffer (100/300/1 mM: pH 7.6 at melting ice temperature). Prolonged or repeated extraction for up to 50 min. did not increase extractable activity. Aliquots (15µl) from the supernatant (10000 g, 8 min., and 4°C) were introduced into a 2- step assay (formation of UDPG by the enzyme: specific step; determination of the product UDPG via UDPG dehydrogenase: indicator step). The specific step was performed in 96-well microtiter plates (0.3 ml cuvette volume), the reagent contained in a total volume of 150 µl, 20 mM Hepes-KOH pH (6.7) and 0.5M sucrose.

The reaction (4 parallel each) was initiated with UDP (10mM final concentration). Blanks were run with either doubly distilled water instead of UDP, or with sample-free extraction buffer. The plates were incubated at 30°C. After 60 min. the reaction was terminated by heating the plates in a dry bath (100°C, 15 min.). When back to ambient temperature, 60 µl of indicator reagent (0.5M glycine-KOH, pH 8.9; 2mM NAD) was added. After reading the extinction (E_o; 340nm; microplate reader 340ATTC, SLT, Salzburg, Austria), the reaction was started by adding 10mM UDPG dehydrogenase. The reaction was controlled by reading the plates every minute using the software package supplied with the instrument. This software allowed for continuous data acquisition and the visualization of the kinetics of each single well in parallel on a PC monitor. The incubation was terminated when all readings reached a steady state which was after approximately 30 min.

Invertase:

To be able to better evaluate the importance of SS we also assayed acid Invertase activity. Sample material (as mentioned above) was extracted for 5 min. with 1 ml ice-cold Tris/Borate buffer (100/300: pH 7.6) containing 5% (w/v) soluble PVP (MW 10000). Activities were assayed in microtiter plates with both 10000g pellets and supernatants (8 min., 4°C). The assay contained in a total volume of 150 µl Citrate/Phosphate buffer (100/100 mM; pH 3.8), sucrose (200mM) and 15 µl extract. Incubation was for 90 min. at 30°C. The reaction was terminated by addition of NaOH (final pH about 6.5) and keeping the plates at 100°C for 15 min. in a dry bath. Glucose and fructose formed were quantified according to Jones and Outlaw (1981). Protein content was determined by the method of Bradford (1976) with BSA as the slandered protein. All enzyme activities were calculated and expressed as micromoles of product formed per gram of total protein per min.

RESULTS AND DISCUSSIONS

Results:

Tissue Extraction:

From a range of buffers tested the combination of Tris/Borate yielded the highest relative rates of enzyme activities. Variation of Tris/Borate ratios between 5 and 0.2 showed no further improvement of recovered activity (data not shown). Addition of surface-active, phenol-binding compounds such as polyvinylpyrrolidone was essential to increase extractable activity. However, the equipments were different for both enzymes. SS responded in a concentration dependent manner toward insoluble PVPP, yielding highest activities at approximately 10%, w/v (Fig. 1). Extraction in the presence of soluble PVP, in contrast, resulted in lower levels of activity. An example of an on-line microplate reader assay of this enzyme is shown in Fig. (2).

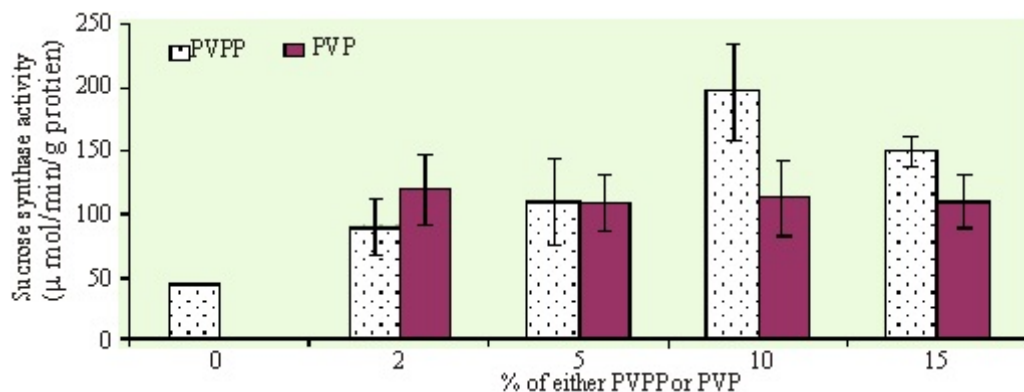


Fig. 1: Effect of phenol-binding polyvinylpyrrolidone (PVPP, insoluble; PVP, soluble) on the extractable activity of sucrose synthase of wheat.

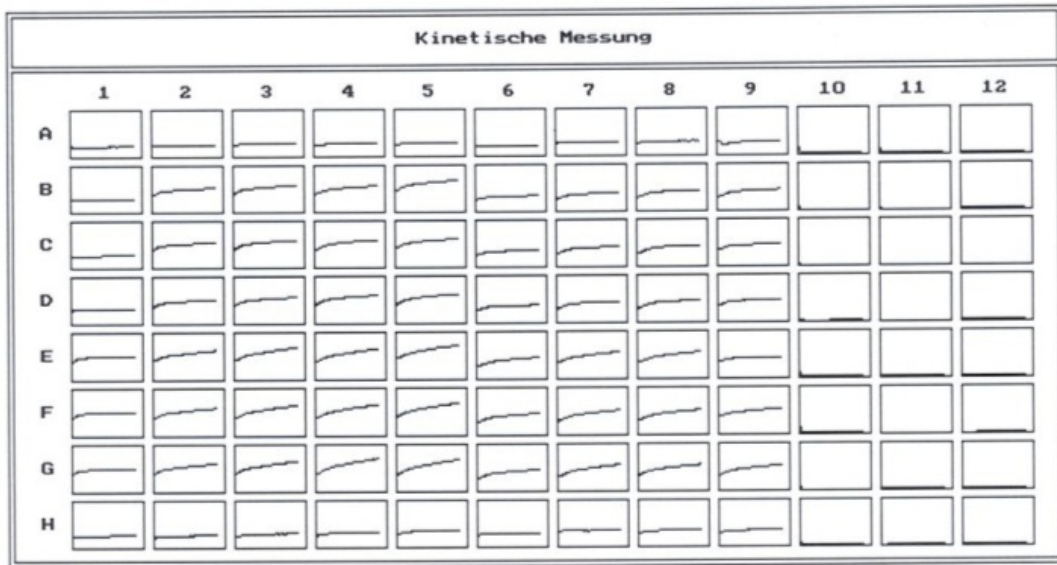


Fig. 2: Example for a microplate reader printout. The kinetics represent a SS assay. Rows A to D background (unspecific) rates: extracts without substrate UDPG; Rows E to H: complete assay; column 1 no leaf extract added. Time of incubation was 60 min.

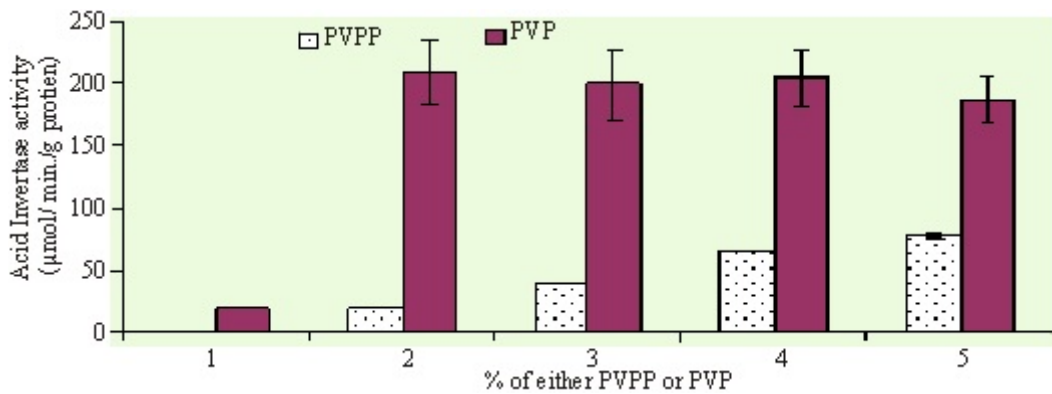


Fig. 3: Effect of phenol-binding polyvinylpyrrolidone (PVPP, insoluble; PVP, soluble) on the extractable activity of acid invertase of wheat.

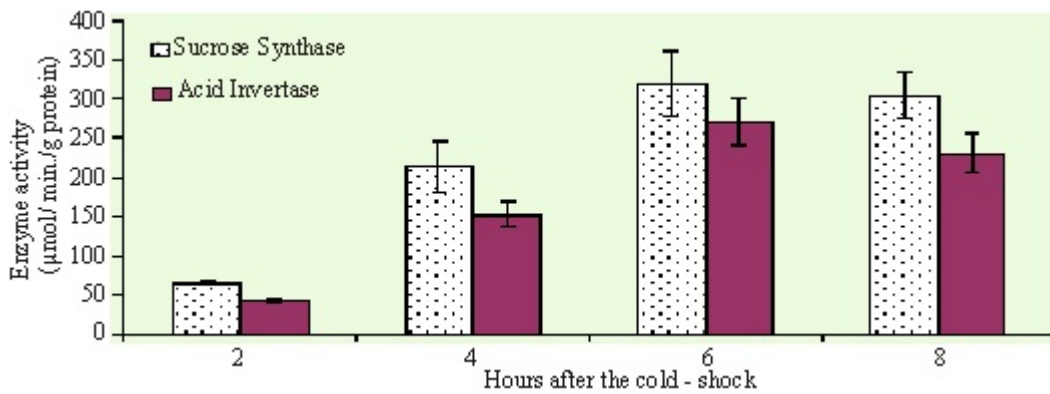


Fig. 4: Changes of extractable activities of sucrose synthase and acid invertase during cold chilling of wheat.

Invertase, on the other hand, required soluble PVP for maximum activity (Fig. 3). Sucrose synthase and acid invertase activities have been shown to increase when wheat plants were submitted to a cold shock. Fig. (4) summarizes apparent activities of the enzyme extracted from wheat leaves 2, 4, 6 and 8 hours after cold acclimation. The amount increase reached three to five folds when plants were acclimated for 6hr. (comparing with the control plants). A similar increase in SS activity was reported by Calderon and Pontis (1985). Tognetti *et al.* (1989) have shown that wheat plants acclimated at 4°C return to the metabolic levels they had prior to the cold shock when plants were moved back to room temperature.

Discussion:

This communication deals with the refinement of current methods for the assay of sucrose synthase and acid invertase in wheat using a microtiter plate system as well as the determination of both enzyme activities during cold-shock of one week old wheat seedlings. Exposure of plants from temperate and cold climatic zones to chilling temperatures leads to an accumulation of fructans (Pollock and Ruggles, 1976; Jones and outlow, 1981; Pontis and Campillo, 1985) which is preceded by an increase in the level of sucrose in the plant cell. This increment may be caused by the low demand for photosynthates at low temperature. During growth under these conditions, the metabolism undergoes an adaptation process. When wheat seedlings were exposed to chilling temperature (4°C) the activity of both SS and invertase started to increase 4 hours after the beginning of the stress. This increase could be connected with the transport of sucrose to the vacuole where it is used for fructan synthesis or it accumulates as sucrose (Kandler and Hopf, 1982; Einig *et al.*, 1991).

This work presents evidence that during cold acclimation, SS and acid invertase levels rises. With regards to the assay, our data clearly show that it is possible to extract both enzymes with good activities from wheat seedlings. The actual rates are in a range reported for other plant tissues (Hubbard *et al.*, 1989), and the use of a microplate reader system greatly increased sample throughput at improved reproducibility.

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