Plant Growth Regulation **35:** 81–91, 2001.

The effect of water stress on the activities of key regulatory enzymes of the sucrose to starch pathway in wheat

A. Ahmadi[1] and D.A. Baker[1,*]
[1]*Department of Biological Sciences, Wye College, University of London, Wye, TN25 5AH, Ashford, Kent, UK;* [2]*Department of Agronomy and Plant Breeding, College of Agriculture, University of Tehran, Karaj, Iran;* **Author for correspondence (e-mail: dennis.baker@ic.ac.uk; phone: +44 1233 812401; fax: +44 1233 813140)*

Received 11 August 2000; accepted in revised form 3 November 2000

Key words: Enzyme, Grain filling, Starch, Sucrose, Water stress, Wheat

Abstract

Developmental changes in the starch and sucrose content of grains and the activities of enzymes of starch synthesis in wheat were studied under water stress conditions. Water stress caused a marked reduction in the sucrose and starch content of the grains. Sucrose synthase (SS) and UDP-glucose pyrophosphorylase (UDP-Gppase), showed higher catalytic activity and more resistance to water stress compared with amyloplastic enzymes. ADP-glucose pyrophosphorylase (ADP-Gppase) activity was reduced to a low level under both *in situ* and osmotic stress conditions in which grains failed to accumulate dry matter *in vivo*. Granule-bound starch synthase (GBSS) also responded rapidly to *in situ* water stress treatments as did ADP-Gppase. Reduction in GBSS activity at the time of growth cessation *in situ* was less than that of ADP-Gppase and the enzyme did not respond to severe osmotic stress. Soluble starch synthase (SSS) was the enzyme most sensitive to water stress in that it responded earlier, and to a greater extent, than the other enzymes. However, under severe dehydration conditions, leading to cessation of growth, the decline in SSS activity was less than that for ADP-Gppase. SSS showed the lowest *in vitro* activity followed by GBSS. These results suggest that SSS is the site of response to water stress by which the rate of grain growth can be affected, whereas growth cessation is due mainly to the inactivation of ADP-Gppase.

Abbreviations: ADP-Gppase – adenosine diphosphate glucose pyrophosphorylase, ES – ethanol soluble, EINS – ethanol insoluble, UDP-Gppase – uridine diphosphate glucose pyrophosphorylase, SS – sucrose synthase, SSS – soluble starch synthase, GBSS – granule-bound starch synthase

Introduction

Under adequate growing conditions, both rate and duration of grain growth (starch deposition) during grain filling are determined mainly by factors that operate within or close to the grain itself (Jenner et al. 1991). The rate of dry matter accumulation (*i.e.* import) is determined by sink strength which is a product of sink size and the metabolic activity of the sink organ during development (Ho 1988). Biochemical conversion of sucrose to starch is one of the most important components of sink strength and can be determined by the catalytic activities of one or more of the enzymes involved in this pathway. The relative importance of any one of these enzymes in this respect may be dependent on plant type. The enzyme ADP-Gppase is one of the enzymes which is believed to be the primary site of regulation of starch deposition in storage tissue (Priess 1991), and is regarded as a possible determinant of sink strength (Doehlert 1993). In wheat endosperm, however, it is the maximum catalytic activity of SSS, but not ADP-Gppase, which is thought to be the important factor controlling the rate of synthesis of starch (Jenner and Hawker 1993; Keeling et

al. 1993). SS activity is positively related to dry matter accumulation in tomato fruits (Demnitz-King et al. 1997) and the activity of this enzyme was reported to be higher in wheat kernels achieving greater maximum dry weight (Dale and Housley 1986). The duration of SS activity is believed to be important in determining the duration of grain filling (Chevalier and Lingle 1983).

Water stress during grain development causes large yield losses in cereals including wheat. This reduction is mainly accounted for by a reduction in starch accumulation, since in general over 65% of cereal dry weight is accounted for by starch (Duffus 1992). In those experiments in which reduced final yield (*i.e* starch deposition) under water stress conditions was not accompanied by a lack of supply (Brooks et al. 1982; Westgate 1994) and reduced endosperm cell and starch granule number (Brooks et al. 1982) clearly impairment of one or some of the sink activity components, (*e.g*. starch synthesis processes) could have been responsible for these reductions. The activity of many enzymes in the starch biosynthesis pathway declines as grains approach maturity when the grain water content reaches a very low value (Chevalier and Lingle 1983; Doehlert et al. 1988; Ou-Lee and Setter 1985). Decline in *in vitro* starch synthesis in osmotic stressed potato disks was thought to be due to a direct effect of stress on one or more of the reactions in the pathway of sucrose to starch conversion (Geigenberger et al. 1997). Water stress during early grain development in maize resulted in a reduction in starch content and GBSS activity of the kernels (Ober et al. 1991). Other workers have demonstrated that GBSS from wheat grain was fairly stable under varying conditions, of *in vitro* assay (Caley 1986) and reduced grain water content (Caley et al. 1990). A brief period of water stress during meiosis of pollen mother cells in wheat severely reduced the activity of acid invertase and to a lesser extent SS (Dorion et al. 1996) whereas the activity of starch synthesis enzymes (SSS, GBSS and ADP-Gppase) was not affected significantly. However, in a similar experiment in rice plants, the activity of SSS and acid invertase followed by ADP-Gppase (to a lesser extent) was reduced by stress but SS activity was not affected (Sheoran and Saini 1996).

The effect of temperature on sink metabolic activity has been extensively investigated. A decline in SSS activity of heated grains of wheat was highly correlated with the rate of starch synthesis (Hawker and Jenner 1993; Jenner et al. 1993; Keeling et al. 1993). The activity of other key enzymes of starch synthesis (SS, GBSS, ADP-Gppase, UDP-Gppase) in wheat was not readily affected by the range of temperature treatments used in these studies. However, these results are not universal for other plant types or even all experimental conditions and there are other reports implying enzymes other than SSS as regulatory sites for starch synthesis under stress conditions. The activity of GBSS was reduced under high temperature, which was consistent with a reduction in starch content (Caley et al. 1990). In maize kernels cultured *in vitro*, among the several enzymes of starch synthesis assayed, only ADP-Gppase showed a marked response to heat stress (Duke and Doehlert 1996). In other studies reduction in grain weight in response to heat stress was due to reduced SS (MacLeod and Duffus 1988) or invertase (Cheikh and Jones 1995) activities.

Despite extensive studies on the effect of heat stress on the enzyme activity related to starch synthesis in cereals, the effect of water stress has gained little attention and there are no available data on the effect of water stress on enzymes of starch synthesis in wheat. In the study reported here the effect of different water stress regimes on five key regulatory enzymes of starch synthesis has been investigated during grain development. To eliminate any water stress-induced reduction in sink capacity (endosperm cell number), the timing of water stress was chosen so that it did not interfere with the cell division processes.

Material and methods

Growth conditions

Spring wheat plants (*Triticum aestivum* var. Cadenza) were grown in a glasshouse under natural light supplemented to provide 300 μmol m^{-2} s^{-1} photosynthetic active radiation (PAR) at plant height by 400 W mercury vapour lamps extending the light period to 16 h. Plants were watered twice daily and supplied with 1 g l^{-1} Sangral 211 fertiliser weekly through watering. The plants were transferred to a growth chamber at the early jointing stage. Air temperature was held at 20/15 °C during the light/dark period and air relative humidity was 60 to 70%. A photoperiod of 16 h was obtained with a mixture of 400 W Sodium and Halide lamps supplemented with 25 W Tungsten lamps providing 850 μmol.m^{-12} s^{-1} PAR at ear level. Plants were watered daily to near field ca-

pacity and supplied with 1 g l^{-11} Sangral 211 fertiliser twice weekly through watering. In all experiments in this work plants within each treatment were divided in a manner that minimized variations in plant materials between treatments and sampling stages.

Stress imposition

The imposition of *in situ* water stress commenced at 10 d.a.a to reach 15% FC (referred to here as early mild stress) or at 15 d.a.a to reach 10% FC (referred to as severe late stress) a few days later. Water was withheld from treated pots and the soil water content allowed to fall to 10 or 15 % of FC and the pots then weighed every day. Sufficient water was applied on each occasion to return the soil moisture to these original levels. In control treatments the soil water status was maintained at 50% FC by weighing the pots every day and adding sufficient water to bring the soil moisture to its original value. Preliminary experiments showed that compost moistures above 15% had no detrimental effects on the plants. The stress levels experienced by the plants (RWC and water potential) were not determined. Unless otherwise stated, two treatments of control and early mild stress with four replications were employed.

Osmotic stress was induced employing a detached ear culture system. Wheat plants were grown as described above. At 18 d.a.a. uniform ears were cut below the penultimate node and divided into groups (treatments) of four replicates. The flag leaves were removed, the stem of the detached ears surface sterilised with 10% sodium hypochlorite and recut under sterilised distilled water 2 cm below the flag leaf node (Singh and Jenner 1983). The explants were then placed in sterilised 10×2.5 glass vessels containing culture medium (Donovan and Lee 1977) and sealed with cotton wool. In the culture medium glutamine (0.02 M) was used as the sole source of nitrogen and the sucrose concentration was 40 g l^{-1} (Barlow et al. 1983). The cultured ears were maintained at 20/15 °C day/night temperature in a controlled environment cabinet with air relative humidity of 55 to 60%. A photoperiod of 16 h was provided by a mixture of Sodium and Tungsten lamps providing 180 μmol·m^{-2} s^{-1} PAR at ear level. The media vessels were covered with aluminium foil and, to reduce microbial contamination, immersed in a shallow water bath system maintained at 3–5 °C. Ears were cultured for 10 days (from 18 to 28 d.a.a). Media were changed after 5 days and consumption measured volumetrically. Super grade Polyethylene glycol 8000 (PEG 8000) with minimum heavy metal contamination (Sigma ultra Sigma Chemical Ltd) was added to the culture medium at 6 %. Preliminary experiments showed that this concentration in the culture medium caused a marked reduction in grain water content.

Sucrose and starch assays

A separate experiment, consisting of two treatments of control and early mild stress (*in situ*) was conducted to determine developmental changes in starch content. Grains were sampled at 16, 24 and 32 d.a.a. from the middle of each ear and divided into two groups. One group was used for grain water content determination and another set was frozen in liquid nitrogen and kept at −20 °C for starch assays. Grains (5 per assay) were first extracted in 80% ethanol, rinsed with cold water, blotted dry and then used for starch determination. Starch was extracted and assayed following published procedures (Ahluwalia and Ellis 1984; Caley et al. 1990). Glucose released from starch hydrolysis was determined spectrophotometricaly using an SP6-550 UV/vis Pye Unicam spectrophotometer. Readings were converted to mg. starch by means of a standard curve. Blanks were run without being digested with a-amylase and amyloglucosidase to measure contaminating glucose. For the standard curve, samples of 50, 100, 200, and 300 mg unmodified wheat starch (Sigma Co. Ltd) were added to 10, 20, 40 and 60 ml 0.05 M perchloric acid (respectively), mixed well, and then heated for 30 min at 97 °C. When cool, samples were made up to 100 ml with deionised water and then treated as described for main samples.

For the sucrose assay, grains (five per assay, see below) were halved transversely and extracted with 80% ethanol at 80 °C three times each for 1.5 h. The extracted grains were rinsed with additional 80% ethanol, washed with water, blotted dry and kept at −20 °C for starch determination. The extracts were combined, evaporated at 40 °C in an air-circulating oven and used for the sucrose assay. Enzymatic assays for sucrose were performed using the method described below (Jones et al. 1977):

Enzyme extraction and assays

Sampling

Three treatments, control, early mild and late severe stress, were employed. Within each treatment plants

were sub-divided into two sub- groups of four and eight, the former sampled at day 15 and the latter at 25 and 32 d.a.a. In the first group grains were removed from the middle of each spike, mixed and then divided into groups of five, frozen in liquid nitrogen and kept at −80 °C. In the second group, grains were carefully removed from each paired plant at day 24 and a corresponding set of grains sampled at day 32, and treated as above. These grains were used for enzyme and sucrose assays, grain dry weight and water content estimations. The relative water content obtained was used to estimate the dry weight of grains used for enzyme assays.

In ear culture experiments (severe osmotic stress condition), grains from one side of the ears were sampled at the end of the culture period and kept for enzyme assay as above. Grains from the opposite side of the spike were used for grain dry weight and water content estimation. Five grains were used for SSS and GBSS assays and five for SS, ADP-Gppase and UDP-G ppase activity.

Extraction and assays

Grains (5 per assay) were weighed and homogenised with a pestle and mortar in 0.5 ml ice-cold extraction medium (EM) as described by Hawker and Jenner (1993). The supernatant was carefully decanted into an Eppendorf tube and kept on ice for immediate assay of all enzymes except the granule bound starch synthase. For the latter enzyme, the pellet was resuspended in 1.5 ml EM, mixed well and then filtered through two layers of muslin (Caley et al. 1990). Debris remaining on the muslin was rinsed with an additional 2 ml of EM. The filtrate was centrifuged at 10000 g at 0 to 4 °C for 10 min. The supernatant was removed and the pellet resuspended in 1.5 ml EM, mixed and re-centrifuged as before. The final pellet was resuspended in 1.0 ml of EM, kept on ice and used for immediate assay.

SSS (EC 2.4.1.21) was assayed using the anion exchange procedure for removing unreacted ADP[^{14}C] glucose (Jenner et al. 1994). The activity of the enzyme was measured by determining the amount of radiolabel incorporated into starch from ADP [^{14}C] glucose. 1.5 ml cocktail T with a Toluene/ Triton x-100 base along with 400 ml water (to gelatinise the scintillant) were added to the column eluate collected in plastic bottles. The incorporated [^{14}C] ADP-glucose was determined by liquid scintillation spectrometry. Samples were counted for 10 min and corrected for counting efficiency. The activity of granule-bound starch synthase (GBSS, ADP glucose- a -(1,4)-glucan-a-4-glucosyltransferase, EC 2.4.1.21). was assayed by incubation of the enzyme preparation obtained from washed precipitate as described for soluble starch synthase. However, the reaction was terminated with cold methanol-KCl and starch was precipitated by the addition of solid carrier starch and centrifugation. The pellet was washed twice with EM and distilled water and the final pellet resuspended in distilled water and counted as described for SSS. SS. (UDP-glucose: D-fructose 2- a glucosyltransferase EC 2.4.1.13) activity was determined employing the method of MacLeod and Duffus (1988). The assay was carried out in the direction of the sucrose-dependent UDP-glucose formation in the presence of UDP. ADP-Gppase activity (ATP: a-D-glucose-1-phosphate adenylyltransferase, EC 2.7.7.27) was assayed based on the method of Riffkin (1987). Enzyme activity was determined spectrophotometrically by incubating ADP-glucose and PP_i with the supernatant enzyme preparation and measuring the production of glucose-1-phosphate in a coupled reaction. All assays were conducted over a range of enzyme concentrations and reaction times during which the increase in absorbancy (for SS, ADP-Gppase, and UDP-ppase) or [^{14}C] sucrose incorporation into starch was linear.

Results

Grain starch and sucrose content

During the early stage of water stress, up to 16 d.a.a, starch content of the grains was unaffected by stress and showed a slight reduction at day 24 compared with their respective control. At day 32 (22 days drought) however, the starch content of stressed grains was reduced significantly to 74 % of the control and was accompanied by a marked reduction in grain water content (Figure 1). Water stress also caused a significant reduction (22%) in grain sucrose content measured at 16 and 24 d.a.a. and a greater reduction of 48% at 32 d.a.a. (Figure 2).

Enzyme activities

In situ water stress

The changes with time in the pattern of enzyme activity are expressed on an endosperm unit basis, fresh weight and dry weight basis. Although changes on a fresh weight basis are a conventional way of express-

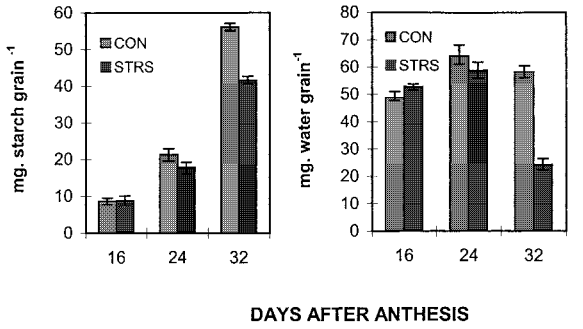

Figure 1. Changes in wheat grain starch, water content under control (CON), water stress (STRS) conditions. Water was withheld from 10 d.a.a to give 15% FC. Data are means of four replicates. Bars indicate SEM

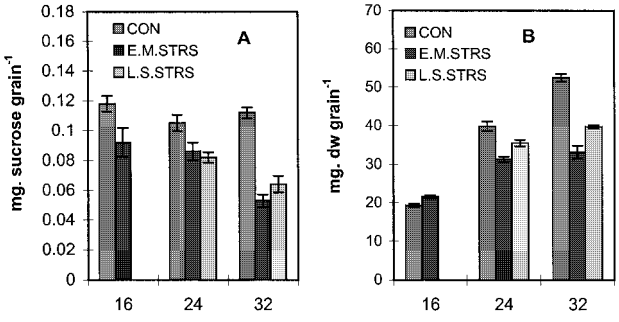

Figure 2. Changes in sucrose content (A), dry weight (B) of wheat grains corresponding to those used for enzyme assays under control (CON), water stress conditions. Water was withheld from 10 d.a.a to give 15% FC. (E.M.STRS) or from 15 d.a.a to give 10% FC. (L.S.STRS). Data are means of four replicates. Bars indicate SEM.

ing results, the pattern can be the result of changes in the fresh weight of the grain. As the fresh weight is grain dry matter and water, and the latter is considerably decreased under water stress conditions, grain dry weight was used to eliminate this difference and relate the enzyme activity to the unit of sink mass. Grain unit and grain dry weight basis were considered as two important criteria to indicate the degree of stress effects on this sink (see Discussion).

SS activity per grain, measured at day 16 and 24, was not affected by early mild water stress starting from day 10 (Figure 3). When stress was prolonged to 22 days (32 d.a.a.), a decline of 33% was observed in the enzyme activity per grain of stressed plants compared with the controls. This difference, nonetheless, was not apparent on a fresh or dry weight basis. Under late, severe stress conditions starting from 15 d.a.a. enzyme activity of stressed grains measured at

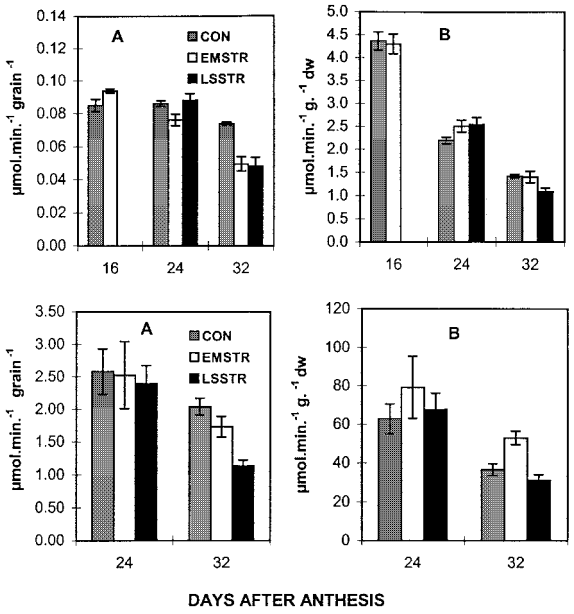

Figure 3. Effect of long term water stress on SS (top panels), and UDP-Gppase (bottom panels) activities of wheat grains expressed on a grain (A), and grain dry weight (B) basis. See (Figure 2) for other details.

day 24 was not affected by this treatment. Longer periods of stress (at 32 d.a.a.) resulted in a reduction of 32% in the enzyme activity of stressed grains compared with that of the control, on both a grain and grain dry weight basis (Figure 3).

The activity of ADP-Gppase and UDP-Gppase in stress treatments was only assayed at days 24 and 32. UDP-Gppase activity was not affected by either stress treatment at day 24. At day 32 a small reduction of 11% on a grain basis was observed under early mild stress. Under severe stress (l.s.strs), however, the activity of the enzyme per grain, measured at day 32, fell to 55% of the control value (Figure 3).

A period of 15 days mild stress from 10 to 24 d.a.a caused a significant reduction in ADP-Gppase activity (44% and 24% per grain unit and dry weight basis respectively) (Figure 4). Late severe stress had no effect on enzyme activity at day 24. By day 32, however, the enzyme activity in stressed grains showed a substantial reduction of 88% and 92% in comparison with that of the control under early mild and late severe stress treatments respectively. The reduction on a dry weight basis was 80% and 86% of the control under early mild and late severe stress regimes respectively (Figure 4).

Among the enzymes of starch biosynthesis assayed in this work, the starch synthase enzymes, particularly

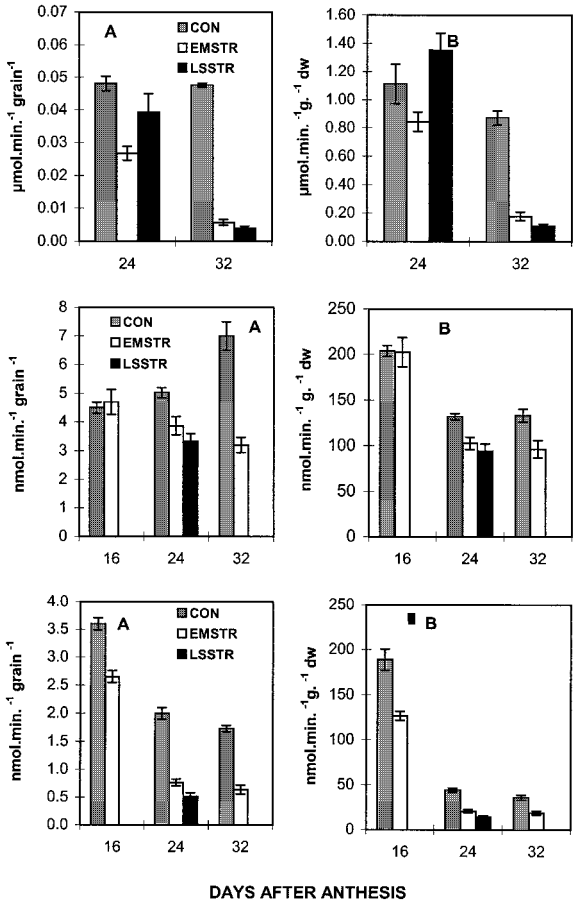

Figure 4. Effect of long term water stress on ADP-Gppase (top panels), GBSS (middle panels), and SSS (bottom panels) activities of wheat grains expressed on a grain (A), and grain dry weight (B) basis. See (Figure 2) for other details

the soluble form, were more responsive to water stress than the other three enzymes (Figure 4). The activity of these two enzymes was assayed at days 16, 24 and 32 under early mild stress and at day 24 a late severe stress treatment was also included. A six day period of early mild stress from 10 to 16 d.a.a. did not have any effect on the GBSS activity. When the stress period was prolonged to 24 d.a.a. the enzyme activity displayed a 22% reduction on both a grain unit and grain dry weight basis compared with that of the control grains. At day 32 (a 22 day water stress period), enzyme activity showed a significant reduction on the basis of all units. The effect of late severe stress conditions occurring between days 15 and 24 was more pronounced than mild stress occurring between days 10 and 24. Enzyme activity per endosperm declined to 66% of the control at day 24 (*i.e* 33% reduction) under this treatment and remained significant on a dry weight basis (22 and 28% respectively).

SSS activity was affected markedly even under a short period of mild stress from day 10 to 16 when other enzymes did not show any response (Figure 4). The enzyme activity of stressed grains measured at day 16 was 73 % of the control on a grain basis and 68% of the control on a dry weight basis. At day 24, when early mild stress conditions were prolonged to 14 days, the effect of stress became greater; the enzyme activity of stressed grains fell to 38% of the control ones on a grain basis and 46% of the control on both a fresh and dry weight basis. The same pattern of response to this treatment was observed at day 32. Shorter periods of late severe stress from days 15 to 24 were more effective than longer periods of milder stress occurring between days 10 and 24 in that the enzyme activity under the former condition was significantly lower than that under the latter condition. This pattern of response to late severe stress was also observed when data were expressed on a dry weight basis.

Osmotic stress

Studying the effects of severe and rather prolonged *in situ* water stress on sink metabolic activity (import ability of the sink) is not possible; under these conditions severe dehydration and ultimately desiccation of the leaves would prevent export of assimilates to the grains. This would not allow the effects of such stress conditions on *in vivo* sink activity to be evaluated. Culture of detached ears in a complex medium and addition of PEG to lower the osmotic potential of the medium is one means by which such studies are possible. In this system, sinks (grains) may experience a different degree of water stress without being under severe source limitation.

Enzyme assays were conducted at the end of culture periods when grains from stressed ears had experienced a 10 day dehydration. SS activity of grains from PEG treated ears showed a significant reduction (46%) compared with that of control grains (Figure 5). This difference was not apparent when calculations were made on a grain and dry weight basis due to a substantial reduction in grain dw (Figure 5). UDP-Gppase of stressed grains showed a substantial reduction (68%) in comparison with grains from control ears on a grain unit basis and the reduction was still significant when enzyme activity was calculated on a dw. basis (48% reduction) (Figure 5). The activity of the ADP-Gppase in grains of stressed ears

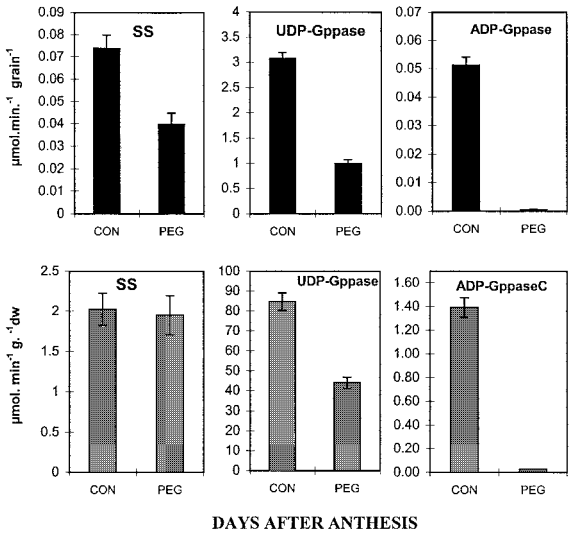

Figure 5. Effect of reduced water uptake by detached ears of wheat on SS, ADP-Gppase, and UDP-Gppase activities, expressed on a grain (top panels), and grain dry weight (bottom panels) basis. Ears were detached from well-watered plants at 18 d.a.a, cultured in a liquid medium without PEG (CON) or containing 2.5% PEG 8000 in the first half of the culture period followed by 6% PEG in the second half. Data are means of four replicates. Bars indicate SEM.

dropped to an almost undetectable level (99% and 98 % reduction on a grain basis and dw basis respectively) compared with their respective controls (Figure 5). The enzyme GBSS showed a pattern of response similar to that of SS in that the decline in enzyme activity was significant ($p < 0.05$) only on a grain unit basis and the difference between treatments was not apparent on a dw basis (Figure 6). The soluble form of the starch synthase enzyme appeared to be in a second order of sensitivity to this type of stress after ADP-Gppase. The enzyme activity displayed a dramatic reduction of 74% on a grain basis, and 45% reduction on a grain dw. basis (Figure 6).

Discussion

Grain starch and sucrose content

Water stress caused a marked reduction in grain starch content at 32 d.a.a. Results from a parallel experiment (data not presented) indicated that growth of stressed grains ceased at about this stage which was also coincident with a rapid reduction of grain water content. Since the partitioning of carbon into starch reserves depends on assimilate supply as well as demand, the decreased starch level could have been due

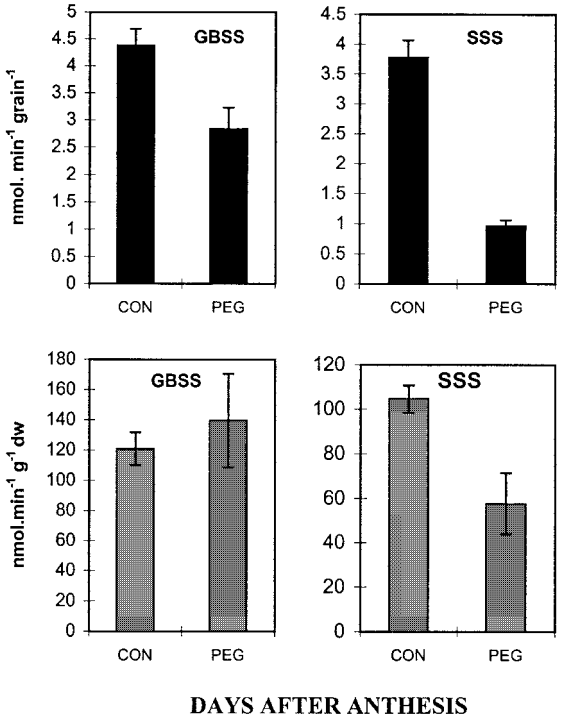

Figure 6. Effect of reduced water uptake by detached ears of wheat on GBSS, SSS activities expressed on a grain (top panels), and grain dry weight (bottom panels) basis. See (Figure 5) for other details.

to either inadequate photoassimilate supply during the period of intense reserve accumulation or to a direct impairment of the starch synthesis machinery as a result of sink dehydration. A marked reduction in grain sucrose content raises the possibility that at least part of the reduced starch deposition could have been due to limited substrate for metabolic processes during the active grain fill period. This is consistent with the results of previous studies in which reduced assimilate supply has been taken as the main cause of yield loss (Barlow et al. 1983; Kobata et al. 1992; Westgate et al. 1989). A reduced sucrose level could affect sink metabolic activity both directly (substrate limitation for metabolic processes) and indirectly (coarse control, to be referred to later). However, other investigators have shown that reduced grain growth under water stress conditions (Brooks et al. 1982; Westgate 1994) or cessation of growth under control conditions (Brooks et al. 1982; Jenner et al. 1991; Lingle and Chevalier 1984) was not due to lack of assimilates. In these cases reduced potential sink activity was the most likely cause of reduced/cessation of growth. This assumption is also consistent with the results of *in vitro* studies (parallel experiments, data not pre-

sented here) in which the *in vitro* starch synthesis ability of *in situ* water stressed grains was considerably lower than that of control ones.

Enzyme activities

In this study, changes in enzyme activity have been expressed relative to the change in dry weight of parallel grains from the same ear (see Materials and methods). The activity of the enzymes was assayed during the linear phase of grain fill in control plants and the last sampling was coincident with growth cessation in stressed grains. This timing of sampling allowed detection of whether growth cessation was related to the diminished activity of the enzymes studied. Results expressed on a grain basis indicated significant adverse effects of stress on enzyme activity while expressing data on a dry weight basis made such effects less apparent. The timing of water stress was such that it did not interfere with the active cell division period and thus endosperm cell number (sink capacity). Under these conditions reduced *in vitro* enzyme activity per grain could not be attributed to reduced endosperm cell number; rather it would imply reduced enzyme content per endosperm cell. Thus, while results on a grain basis will be taken here as an indication of stress effects on enzyme activity, data on a dry weight basis will be considered to indicate the importance of the enzyme in limiting the rate or cessation of growth.

The unresponsiveness of SS to water stress during the earlier stage of water stress in this work is in agreement with previous observations on maize kernels during early grain fill (Ober et al. 1991) and wheat and rice anthers during pollen mother cell division (Dorion et al. 1996; Sheoran and Saini 1996). SS activity per grain was reduced at a later stage of stress coincident with cessation of grain growth. Studies on wheat and barley under both normal (Chevalier and Lingle 1983) and heat stress (MacLeod and Duffus 1988) conditions suggest that the duration of SS activity may be important in determining the duration of grain fill.

The average catalytic activity of UDP-Gppase was substantially higher than that of the other enzymes assayed in this study implying that the rate limiting step is unlikely to be related to this enzyme; a substantial level of enzyme activity even at a very late stage of grain fill (55 d.a.a) was reported in wheat grain (Riffkin 1987). In the present work, even under stress conditions in which grains failed to accumulate dry matter *in vivo*, the UDP-Gppase showed considerably higher catalytic activity than other enzymes under similar conditions and at the same stage, ruling out the possibility that this enzyme was rate-limiting, even under severe stress conditions.

The average ADP-Gppase activity was nearly 48 times lower than that of the UDP-Gppase. The considerably lower activities of ADP-Gppase than both SS and UDP-Gppase observed in the present work may imply that ADP-Gppase is one potential site of rate limitation, in agreement with the view that this enzyme is the primary site of regulation of starch deposition in storage tissues (Doehlert 1993; Priess 1991). Compared with SS and UDP-Gppase, ADP-Gppase appeared to be more responsive to water stress. The sensitivity of this enzyme to water stress was previously reported in wheat and rice anthers (Dorion et al. 1996; Sheoran and Saini 1996), potato tuber discs cultured *in vitro* (Geigenberger et al. 1997) and wheat (Caley et al. 1990). Much of the reductions in maize grain cultured *in vitro* under heat stress conditions was proposed to be due to a decline in ADP-Gppase activity which corresponded with the message levels for its sub units (Duke and Doehlert 1996). An important feature of ADP-Gppase is its allosteric properties through which the enzyme regulates starch biosynthesis in plant tissues and a possible mechanism by which stress affects the activity of this enzyme, could be through allosteric effects on enzyme activity. In potato tubers the decreased level of 3 PGA was correlated with a decrease of ADP-glucose, the product of ADP-Gppase and under severe stress a decline in ADP-glucose was proposed to be due to an inhibitory effect of increased Pi concentration, as a result of cell dehydration, on ADP-Gppase activity (Geigenberger et al. 1997). ADP-Gppase of wheat endosperm has been reported to be inhibited by ADP-glucose, the enzyme's product (Duffus 1992). It may be possible that the impaired starch synthase activities, which occurred earlier than ADP-Gppase in the current study, resulted in an accumulation of ADP-glucose, which in turn inhibited *in vivo* ADP-Gppase activity as a result of product inhibition. In severely water-stressed potato, the strong inhibition of starch synthesis was found to be accompanied by a large increase in ADP-glucose (Geigenberger et al. 1997).

At the later stage of *in situ* water stress and under severe osmotic stress, when grains failed to accumulate dry matter *in vivo* (data not presented) the activity of the enzyme showed a substantial reduction, ob-

servations which may imply that ADP-Gppase was involved in terminating starch deposition. None of the other enzymes assayed in this study was inhibited to such an extent at this time (or condition). Inclusion of PEG to the ear culture medium resulted in a cessation of starch deposition and complete inhibition of ADP-Gppase (Caley et al. 1990). A greater reduction in ADP-Gppase activity than other enzymes of starch synthesis towards the end of grain fill, concomitant with a fall in grain water content, has also been reported for maize (Ou-Lee and Setter 1985) and wheat kernels (Riffkin 1987).

Different responses of GBSS to moderate *in situ* and severe osmotic stress treatments (cf Figures 4 and 5) may imply a different mechanism of controlling enzyme activity by different stress conditions. Under *in situ* water stress conditions the slower progress and more prolonged duration of water stress in the whole plant may have invoked alterations in metabolites or other substances outside the sink organs (leaves, roots,) and these substrates may have been transported to the sinks and reduced enzyme performance, whereas in osmotically-stressed detached ears, the presence of such external effects was lacking and sink dehydration was the most predominant factor. The different response of GBSS to these conditions may be explained as GBSS is rather resistant to dehydration conditions while more responsive to external water-induced signals (Caley et al. 1990).

One significant feature of SSS response to water stress was the pattern of response with progression in stress levels. From its earlier response to water stress and thus greater sensitivity of this enzyme to water stress compared with other enzymes, one might expect a substantially greater reduction in the activity of the enzyme during the later stages of *in situ* water stress, where the effects of stress became more evident, or under more severe dehydration conditions provided by PEG treated detached ears. However, this was not the case and the marked reduction in enzyme activity during early *in situ* water stress conditions (at 24 d.a.a.) remained almost the same under more severe dehydration conditions (at 31 d.a.a. *in situ*, and under severe osmotic stress conditions). This pattern of response was not observed for other enzymes. Similar observations have been reported for SSS activity in response to heat stress in wheat (Hawker and Jenner 1993; Jenner et al. 1993; Rijven 1986), where a rapid loss of enzyme activity, observed after an initial period of heating, did not show further reduction (or reduced slowly) by additional periods of heating.

The rate of dry matter accumulation between 16 and 24 d.a.a. was reduced 53% by water stress (Figure 2). Enzyme activities (on a dry weight basis) at day 24 were reduced 55% for SSS, 22% for GBSS and 24% for ADP-Gppase. An 82% reduction in the rate of dry matter accumulation for the period between 24 and 32 d.a.a, corresponded to an 80% reduction in ADP-Gppase activity, a 48% reduction in SSS and a 27% reduction in GBSS activities at day 32. Under more severe stress conditions (PEG treated detached ears) the cessation of dry matter accumulation corresponded to a nearly complete inhibition of ADP-Gppase activity while SSS showed 55% of the activity of the control grains and GBSS was not affected. The catalytic activity of SSS was the lowest among the enzymes assayed in the present study, followed by GBSS and then ADP-Gppase. Several conspicuous features are evident from these observations: (1) among the 5 key regulatory enzymes in the biochemical pathway of sucrose to starch, SSS, GBSSS, and ADP-Gppase are more likely to be affected by water stress, than the other two enzymes. (2) among these enzymes, SSS with the lowest catalytic activity and highest susceptibility, appeared to be a major site of regulation of starch synthesis in the developing wheat grain, and the first enzyme to be affected by stress conditions, and that the control point in the pathway is apparently not associated exclusively with ADP-Gppase or GBSS. (3) whilst SSS activity may limit the rate of starch synthesis, it is the lack of ADP-Gppase activity which terminates grain growth, since the activities of SSS and GBSS were maintained well after starch deposition had ceased.

Although the relationship between dry matter accumulation and enzyme activity can not be equated precisely with the data available, the decline in grain dry weight in the current study was more compatible with the decline in the rate of SSS activity followed by GBSSS than with other enzymes (see above). The average catalytic activity of SSS in control grains during 24–32 d.a.a. (1.69 mg. equivalent ADP-glucose grain^{-1} for 24 h) was close to the rate of dry matter accumulation calculated for this period (1.58 mg grain^{-1} day^{-1}) whereas those of the GBSS and ADP-Gppase were nearly 3 and 27 fold higher (respectively) than the rate of dry matter accumulation. In the present study both grain water content and sucrose level were considerably lower in stressed plants than in controls and at later stages, at about the point where grain filling ceased, there was a rapid water reduction in the grain. Thus reduced enzyme activities

under stress conditions or towards the end of grain fill, can be attributable to one (or both) of these factor(s).

Studying the detailed kinetics of the key enzymes of starch synthesis under water stress conditions can provide more detailed information on the mechanism by which water stress reduces sink metabolic activity. The study of developmental changes in mRNA for key enzymes of starch synthesis, particularly those which are rate limiting or growth terminating, during grain filling up to the end of the grain filling period and determining the fate of developmental mRNA profiles in both control and water stressed wheat kernels would clarify whether the duration of grain filling is controlled at the level of RNA transcription and/or translation. The stimulatory effect of sugar and nitrogen supply on expression of some genes encoding carbohydrate metabolism is documented for other crop plants. The cell-turgor regulation of gene expression in plant organs, other than grains, is also documented and the mechanisms of such sugar-responsive and dehydration-responsive genes are being resolved. Either of these steps (events) can be important targets for further investigations on the effects of water stress on grain filling processes (sink strength).

Acknowledgements

This work was financed by a grant to Ali Ahmadi from the Ministry of Culture and Higher Education of the Islamic Republic of Iran.

References

Ahluwalia B. and Ellis E.E. 1984. A rapid and simple method for the determination of starch and β-glucan in barley and malt. J. Ins. Brew. 90: 254–259.

Barlow E.W.R., Donovan G.R. and Lee J.W. 1983. Water relations and composition of wheat ears grown in liquid culture: Effect of carbon and nitrogen. Aust. J. Plant Physiol. 10: 99–108.

Brooks A., Jenner C.F. and Aspinall D. 1982. Effects of water deficit on endosperm starch granules and on grain physiology of wheat and barley. Aust. J. Plant Physiol. 9: 423–436.

Caley C.Y. 1986. Termination of grain growth in cereals. PhD Dissertation,.

Caley C.Y., Duffus C.M. and Jeffcoat B. 1990. Effects of elevated temperature and reduced water uptake on enzymes of starch synthesis in developing wheat grains. Aust. J. Plant Physiol. 17: 431–439.

Cheikh N. and Jones R.J. 1995. Heat stress effects on sink activity of developing maize kernels grown *in vitro*. Physiol. Plant. 95: 59–66.

Chevalier P. and Lingle S.E. 1983. Sugar metabolism in developing kernels of wheat and barley. Crop Sci. 23: 272–277.

Dale E.M. and Housley T.L. 1986. Sucrose synthase activity in developing wheat endosperms differing in maximum weight. Plant Physiol. 82: 7–10.

Demnitz-King A., Ho L.C. and Baker D.A. 1997. Activity of sucrose hydrolysing enzymes and sugar accumulation during tomato fruit development. Plant Growth Reg. 22: 193–201.

Doehlert D.C. 1993. Sink strength: Dynamic with source strength. Plant Cell Env. 16: 1027–1028.

Doehlert D.C., Min Kou T. and Felker F.C. 1988. Enzymes of sucrose and hexose metabolism in developing kernels of two inbreds of maize. Plant Physiol. 86: 1013–1019.

Donovan G.R. and Lee J.W. 1977. The growth of detached wheat heads in liquid culture. Plant Sci. Let. 9: 107–113.

Dorion S., Lalonde S. and Saini H.S. 1996. Induction of male sterility in wheat by meiotic-stage water deficit is preceded by a decline in invertase activity and changes in carbohydrate metabolism in anthers. Plant Physiol. 111: 137–145.

Duffus C.M. 1992. Control of starch biosynthesis in developing cereal grains. Bioch. Soc. Tran. 20: 13–18.

Duke E.R. and Doehlert D.C. 1996. Effects of heat stress on enzyme activities and transcript levels in developing maize kernels grown in culture. Env. Exp. Bot. 36: 199–208.

Geigenberger P., Reimholz R., Geiger M., Merlo L., Canale V. and Stitt M. 1997. Regulation of sucrose and starch metabolism in potato tubers in response to short-term water deficit. Planta. 201: 502–518.

Hawker J.S. and Jenner C.F. 1993. High temperature affects the activity of enzymes in the committed pathway of starch synthesis in developing wheat endosperm. Aust. J. Plant Physiol. 20: 197–209.

Ho L.C. 1988. Metabolism, compartmentation of imported sugars in sink organs in relation to sink strength. Annu. Rev. Plant Physiol. Plant Mol. Bio. 39: 355–378.

Jenner C.F. and Hawker J.S. 1993. Sink strength: Soluble starch synthase as a measure of sink strength in wheat endosperm. Plant Cell Env. 16: 1023–1024.

Jenner C.F., Denyer K. and Hawker J.S. 1994. Caution on the use of precipitation technique for the assay of soluble starch synthase in crude extracts of plant tissues. Aust. J. Plant Physiol. 21: 17–22.

Jenner C.F., Siwek K. and Hawker J.S. 1993. The synthesis of [^{14}C]starch from [^{14}C]sucrose in isolated wheat grains is dependent upon the activity of soluble starch synthase. Aust. J. Plant Physiol. 20: 329–335.

Jenner C.F., Ugalde T.D. and Aspinal D. 1991. The Physiology of starch, protein deposition in the endosperm of wheat. Aust. J. Plant Physiol. 18: 211–226.

Jones M.G.K., Outlaw W.H., J.R. and Lowry O.H. 1977. Enzymic assay of 10 $^{-7}$ to 10^{-14} moles of sucrose in plant tissues. Plant Physiol. 60: 379–383.

Keeling P.L., Bacon P.J. and Holt D.C. 1993. Elevated temperature reduces starch deposition in wheat endosperm by reducing the activity of soluble starch synthase. Planta. 191: 342–348.

Kobata T., Palta J.A. and Turner N.C. 1992. Rate of development of postanthesis water deficits, grain filling of spring wheat. Crop Sci. 32: 1238–1242.

Lingle S.E. and Chevalier P. 1984. Movement, metabolism of sucrose in developing barley kernels. Crop Sci. 24: 315–319.

MacLeod L.C. and Duffus C.M. 1988. Reduced starch content, sucrose synthase activity in developing endosperm of barley plants grown at elevated temperatures. Aust. J. Plant Physiol. 15: 367–375.

Ober E.S., Setter T.L., Madison J.T., Thompson J.F. and Shapiro P.S. 1991. Influence of water deficit on maize endosperm development. Enzyme activities, RNA transcriptions of starch, zein synthesis, abscisic acid and cell division. Plant Physiol. 97: 154–164.

Ou-Lee T.M. and Setter T.L. 1985. Enzyme activities of starch, sucrose pathways, growth of apical, basal maize kernels. Plant Physiol. 79: 848–851.

Priess J. 1991. Biology, molecular biology of starch synthesis, its regulation. Oxford Sur. Plant Mol. Cell Biol. 7: 59–114.

Riffkin H.L. 1987. The regulation of starch biosynthesis in developing wheat endosperm. PhD Dissertation,.

Rijven A.H.G.C. 1986. Heat inactivation of starch synthase in wheat endosperm tissue. Plant Physiol. 81: 448–453.

Sheoran I.S. and Saini H.S. 1996. Drought-induced male sterility in rice: changes in carbohydrates levels, enzyme activities associated with the inhibition of starch accumulation in pollen. Sex Plant Reprod. 9: 161–169.

Singh B.K. and Jenner C.F. 1983. Culture of detached ears of wheat in liquid culture: Modification, extention of the method. Aust. J. Plant Physiol. 10: 227–236.

Westgate M.E. 1994. Water status, development of the maize endosperm, embryo during drought. Crop Sci. 34: 76–83.

Westgate M.E., Schussler J.R., Reicosky D.C. and Brenner M.L. 1989. Effect of water deficits on seed development in soybean. II. Conservation of seed growth rate. Plant Physiol. 91: 980–985.