Seed Science and Biotechnology ©2009 Global Science Books

Interactive Effects of High Temperature and Phytohormones on Carbohydrate Metabolism in Barley Seedlings

Sondeep Singh* • **Bavita Asthir**

Department of Biochemistry, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana-141 004, Punjab, India

Corresponding author: * **sondeeprai@gmail.com**

ABSTRACT

Germinating seedlings of two barley genotypes with a radicle length of 5 mm were subjected to a brief heat shock (HS) episode of 45°C followed by transfer to a normal temperature (25°C) for five days with and without phytohormones. HS treatment resulted in a decline in the activities of sucrose synthase (synthesis direction) and starch mobilizing enzyme and, thereby, seedling growth. In contrast, acid and neutral invertase activities increased under HS treatment. Exogenous GA_3 (10 μM) partially alleviated the high temperature response by enhancing sucrose metabolizing enzymes while ABA showed antagonistic effects. Soluble sugars showed a synergistic increase under HS and GA_3 treatment but decreased in response to ABA. The sugar supply from endosperm starch mobilization was not a limiting factor for germination but a poorer 'metabolic conversion efficiency' of carbon intermediates, possibly leads to decreased germination rate and hence reduction in crop yield potential.

Keywords: ABA, amylase, GA_3, heat shock, invertase, source-sink relationships
Abbreviations: **ABA**, abscisic acid; **BSA**, bovine serum albumin; **DTT**, dithiothreitol; **EDTA**, ethylene diaminetetra-acetic acid; **GA_3**, gibberellic acid

INTRODUCTION

High temperature (HT) stress is one of the important abiotic stresses of present day studies due to global warming. Both moderately high (15-32°C) and very high temperatures (32-50°C) are frequently encountered in the world's major cropping regimes, particularly in India, South East Asia and West Africa (Russell and Coupe 1994). Recently, HT stress emerged as one of the major constraints of yield losses in cereal-growing regions of the world (Koc *et al.* 2008). At the time of sowing, this results in poor germination of seeds leading to a reduction in crop yield potential (Pagamas and Nawata 2008). Adaptation to the stress is associated with metabolic adjustments that lead to modulation of different enzymes (Shinozaki and Shinozaki 1996). Among these are soluble acid invertase (AI), neutral invertase (NI), sucrose synthase (SS) and total amylase (TA), key regulators of carbohydrate metabolism in barley.

Plant growth regulators (PGRs) have also been found to play an important role in plant responses to stress (Amzallag *et al.* 1992). It is generally accepted that the application of phytohormones to stressed plants may lead to counteraction of adverse effects exerted by stress condition and result in regulation of the plant metabolism and consequently better plant performance (Heikal *et al.* 1982). Little research, however, has been conducted to determine the combined effects of HT and phytohormones on carbohydrate metabolism in barley. The development of HT-tolerant crop plants is an important goal necessary to alleviate future threats to food availability in a rapidly expanding human population. However, this requires comprehensive exploration of the many potential genetic resources and an in-depth understanding of the adaptive mechanism and responses to HT that allow survival in an unfriendly environment.

In view of these findings, the present work was carried out to investigate the interactive effects of high temperature and phytohormones on carbohydrate metabolism in barley seedlings to increase barley productivity.

MATERIALS AND METHODS

Plant material and chemicals

Grains of two barley genotypes, PL 426 (susceptible to leaf blight) and BL 4 (resistant to leaf blight), were procured from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Punjab, India. In earlier study with these genotypes, we have observed that carbohydrate metabolism, especially invertase activities were significantly altered in response to leaf blight disease. HT conditions also favour the development of this disease (Kumar *et al*. 2002). Therefore, in this study, leaf blight susceptible and resistant genotypes were used to explore the responses of carbohydrate metabolism in response to HT.

The fine chemicals and reagents used in this study were purchased from Sigma Chemicals, Corp., St. Louis, USA. All the other chemicals were of analytical grade.

Germination and treatment of grains

Uniform sized grains of each variety were hand-picked and surface sterilized with 0.1% $HgCl_2$ for 1 min followed by thorough rinsing with distilled water. Twenty grains were imbibed in distilled water (20 ml) for 24 h in the dark and germinated at 25°C using Petri dishes (9.0 cm) (Borosil, India) on double layer Whatman No. 1 filter paper moistened with 5.0 ml of either phytohormone, GA_3 (gibberellic acid) or ABA (abscisic acid) (Sigma-Aldrich) (10 μM) or distilled water (control). Three replicates of each genotype were used. A grain was considered to have germinated when its radical emerged at least 5 mm. HS response of different plant species varies with temperature. However, the maximum response was obtained in the range of 40-45°C in many plant species studied (Sridevi *et al*. 1999). So in this study HS at 45°C for 2 h was applied to germinated grains on the first day of germination and thereafter maintained at normal temperature (25°C) for five days as described earlier by Sridevi *et al*. (1999). In the control experiment, grains of both the genotypes were germinated on double layer Whatman No. 1 filter paper moistened

Received: 26 February, 2009. Accepted: 26 May, 2009.

Original Research Paper

with 5.0 ml of distilled water. This set of Petri dishes was maintained at 25°C for five days.

Extraction and estimation of sugars

Free sugars were extracted and estimated from the root and shoot of germinating seedlings after five days of germination according to the method described by Singh and Asthir (1988).

Enzyme assays

Soluble AI (EC 3.2.1.26; pH 4.8), soluble NI (EC 3.2.1.27; pH 7.5), SS (synthesis, EC 2.4.1.13) and TA (EC 3.2.1.1/2) were extracted from fresh tissue samples by the procedures employed by Stommel (1992). The enzymes were extracted with 50 mM Hepes-NaOH buffer (pH 7.5) containing 5 mM $MgCl_2$, 1 mM sodium EDTA, 2.5 mM DTT, 0.5 mg ml^{-1} BSA and 0.05% (v/v) Triton-X100. All these enzymes were assayed from the test extracts as described by Singh and Asthir (1988). In all enzyme assays, the conditions for linear rates with respect to substrate concentration, time, optimum temperature and pH were determined in preliminary assays.

Statistical analysis

The data is presented as mean ± standard error of three replicates. The means of recorded data were compared for significant differences at $P \leq 0.05$ by the Student's *t*-test using GSTAT04 software package.

RESULTS

The results obtained on activity of TA in roots and shoots after 5 days of germination are given in **Table 1**. HS led to a reduction in TA activity in both the tissues compared to seedlings maintained at normal temperature. TA activity in shoots was more sensitive to HS than in roots. Genotype BL 4 showed relatively more reduction in TA activity than PL 426. GA_3 treatment resulted in reversion of TA activity of stressed seedling near to control. Effect of ABA on TA activity was inhibitory at both normal and HT conditions.

Activities of AI and NI in root and shoot are given in **Table 1**. Shoots had higher AI activities compared to roots in both genotypes. In general NI activities were lower than AI activities. Genotype BL 4 had significantly higher AI activities than PL 426 in both root (23%) and shoot (49%). HT treatment resulted in increased activities of both AI and NI. Exogenous application of GA_3 significantly enhanced invertase activities in root and shoot of both genotypes (**Table 1**). In the presence of ABA however, the effect of GA_3 was largely abolished.

In contrast to invertases, SS (synthesis direction) activity was significantly reduced in response to HT (**Table 1**). However, application of GA_3 led to restoration of enzyme activity. In contrast, ABA treatment resulted in reduction of SS activity irrespective of the tissues, temperature and genotype.

Contents of reducing sugar (RS), non reducing sugar (NRS) and total sugars (TS) are presented in **Table 2**. Levels of RS were significantly higher than NRS. Irrespective of the genotypes, RS contents were higher in shoots than in roots consistent with higher invertase activities in shoots (**Table 1**). HS resulted in accumulation of sugars in both roots and shoots. Exogenous application of GA_3 further increased the sugar levels in both genotypes. Effect of ABA on sugar contents was reverse of GA_3 in both genotypes. In stressed seedling, compared to control the observed increase in sugar contents in response to ABA was due to stimulatory effect of HS rather than that of ABA (**Table 2**).

DISCUSSION

The growth of seedlings is dependent upon starch mobilization from the cotyledons and availability of sucrose as a carbon source for meeting the energy demands of the growing tissues. HT reduced the mobilization of starch as indicated by reduced activity of TA (**Table 1**). Higher reduction of TA activity in root and shoot of BL 4 suggested more susceptibility of the genotype to HT stress compared to PL 426. The decreased amylase activity in stressed seedlings results in reduced formation of glucose from transitory starch, thereby leading to a decreased synthesis of sucrose and its reduced supply to root and shoot. This may be responsible for stunted growth under stress condition. The observed reduction in enzyme activity was reversed by GA_3 while ABA effect was inhibitory. Thus GA_3 by stimulating the amylase activity promoted hydrolysis of transitory starch. This consequently leads to enhanced availability of sucrose to the growing axis for its onward utilization to maintain growth and metabolism of seedling under stress condition. Stimulation of gene expression and activity of α-amylase by gibberellins and inhibition by ABA were reported by many authors (Acevedo and Cardemil 1997; Pagano *et al.* 1997). Kaplan *et al.* (2006) also reported that 1-10 μM GA_3 fully reversed the inhibition of amylase activity caused by 10 μM ABA in germinating rice seeds.

Sucrose is invariably the sugar which is transported from cotyledons to embryonic axis during germination. AI is involved in phloem unloading in shoots, thereby converting the sucrose unloaded to apoplast into hexoses and

Table 1 Effect of high temperature and phytohormones (10 μM) treatments on activities of total amylase, acid invertase, neutral invertase and sucrose synthase in root and shoot of barley seedlings. Values are presented as mean ± SE; s/ns, differences in means are significant/non-significant ($P \leq 0.05$) compared to water treatment within the column for a given enzyme; *, differences at 45ºC are significant compared to respective treatment at 25ºC.

Treatment	Root				Shoot			
	PL 426		BL 4		PL 426		BL 4	
	25°C	45°C	25°C	45°C	25°C	45°C	25°C	45°C
Total amylase (μg reducing sugars formed/min/g FW)								
H_2O	13 ± 1.3	7 ± 1.0*	14 ± 1.2	8 ± 1.1*	16 ± 1.4	12 ± 1.0*	19 ± 1.7	13 ± 1.0*
GA_3	15 ± 2.1^{s}	12 ± 1.4*^{s}	16 ± 1.7^{s}	11 ± 1.3*^{s}	25 ± 1.9^{s}	18 ± 1.5*^{s}	24 ± 2.0^{s}	20 ± 2.1*^{s}
ABA	10 ± 1.0^{s}	7 ± 1.1*^{ns}	9 ± 1.4^{s}	6 ± 0.8*^{s}	14 ± 1.5^{s}	10 ± 0.9*^{s}	18 ± 1.3^{s}	12 ± 1.1*^{ns}
Acid invertase (μg sucrose hydrolysed/min/g FW)								
H_2O	113 ± 3.9	133 ± 4.1*	146 ± 4.0	215 ± 5.9*	148 ± 2.8	165 ± 4.8*	291 ± 4.4	317 ± 4.1*
GA_3	155 ± 4.1^{s}	156 ± 3.7^{s}	162 ± 4.0^{s}	223 ± 4.2*^{ns}	175 ± 4.1^{s}	192 ± 4.4*^{s}	310 ± 5.1^{s}	338 ± 5.4*^{s}
ABA	105 ± 3.3^{ns}	121 ± 2.2*^{s}	122 ± 4.3^{s}	184 ± 4.5*^{s}	132 ± 5.2^{s}	150 ± 3.6*^{s}	266 ± 3.1^{s}	279 ± 4.9^{s}
Neutral invertase (μg sucrose hydrolysed/min/g FW)								
H_2O	76 ± 3.1	89 ± 1.5*	84 ± 3.0	105 ± 5.0*	99 ± 1.3	116 ± 3.3*	115 ± 3.1	138 ± 4.5*
GA_3	95 ± 4.1^{s}	116 ± 3.0*^{s}	92 ± 4.1^{ns}	116 ± 4.3*^{ns}	120 ± 3.7^{s}	156 ± 4.1*^{s}	123 ± 2.4^{ns}	150 ± 4.7*^{s}
ABA	69 ± 2.9^{ns}	78 ± 3.5^{s}	71 ± 3.3^{s}	85 ± 2.8*^{s}	87 ± 4.6^{s}	104 ± 3.3*^{s}	92 ± 1.7^{s}	102 ± 2.1*^{s}
Sucrose synthase (μg sucrose formed/min/g FW)								
H_2O	69 ± 2.4	62 ± 1.4	63 ± 2.4	56 ± 1.6	96 ± 2.5	88 ± 3.3*	87 ± 1.4	80 ± 1.2*
GA_3	84 ± 2.2^{s}	72 ± 2.9*^{s}	67 ± 1.5^{ns}	58 ± 1.8*^{ns}	107 ± 2.7^{s}	98 ± 2.6*^{s}	106 ± 1.5^{s}	104 ± 4.0^{s}
ABA	52 ± 2.4^{s}	38 ± 2.0*^{s}	50 ± 1.2^{s}	34 ± 3.2*^{s}	81 ± 2.4^{s}	63 ± 3.6*^{s}	77 ± 1.3^{s}	70 ± 2.7*^{s}

Table 2 Effect of high temperature and phytohormones (10 µM) treatments on levels of reducing, non-reducing and total sugars in root and shoot of barley seedlings. Values are presented as mean ± SE; s/ns, differences in means are significant/non-significant (P≤0.05) compared to water treatment within the column for a given sugar; *, differences at 45ºC are significant compared to respective treatment at 25ºC.

Treatment	Root				Shoot			
	PL 426		BL 4		PL 426		BL 4	
	25°C	45°C	25°C	45°C	25°C	45°C	25°C	45°C
Reducing sugars (mg/g DW)								
H_2O	37 ± 0.9	42 ± 0.9*	40 ± 1.0	44 ± 1.4*	46 ± 1.0	48 ± 1.3	57 ± 1.4	61 ± 1.2
GA_3	43 ± 0.8^s	45 ± 1.0^s	42 ± 1.3^{ns}	46 ± 0.8*^{ns}	51 ± 1.1^s	53 ± 1.5^s	60 ± 1.5^{ns}	64 ± 3.0^{ns}
ABA	35 ± 1.0^{ns}	35 ± 0.6^s	35 ± 1.7^s	42 ± 1.4*^{ns}	43 ± 0.7^{ns}	46 ± 0.8*	52 ± 1.0^{ns}	55 ± 1.1^s
Non reducing sugars (mg/g DW)								
H_2O	27 ± 0.8	29 ± 0.5*	33 ± 0.8	36 ± 0.7*	38 ± 0.7	39 ± 0.9	35 ± 0.8	41 ± 1.4*
GA_3	31 ± 0.4^s	33 ± 0.4*^{s}	34 ± 0.8^{ns}	37 ± 0.9*^{ns}	39 ± 0.9^{ns}	40 ± 0.7^{ns}	36 ± 1.3^{ns}	43 ± 0.7*^{ns}
ABA	24 ± 0.6^s	26 ± 0.7*^{s}	32 ± 0.6^{ns}	35 ± 0.6*^{ns}	35 ± 0.6^s	31 ± 0.7*^{s}	34 ± 0.9^{ns}	39 ± 0.8*^{ns}
Total sugars (mg/g DW)								
H_2O	65 ± 1.4	71 ± 1.0*	73 ± 1.2	80 ± 1.4*	84 ± 1.4	88 ± 1.0*	92 ± 1.6	103 ± 1.4*
GA_3	74 ± 1.5^s	78 ± 1.6*^{s}	73 ± 1.7^{ns}	83 ± 1.7*^{ns}	89 ± 1.1^s	94 ± 1.3*^{s}	96 ± 2.2^{ns}	107 ± 1.2*^{s}
ABA	60 ± 1.3^s	62 ± 1.0^s	68 ± 1.1^s	77 ± 1.5*^{ns}	79 ± 1.3^s	78 ± 1.5^s	86 ± 1.6^s	94 ± 1.1*^{s}

resulting in continued export of sucrose from the phloem (Patric 1990). The observed increase in invertase activities under stress conditions enhanced sink strength and thus provided more reducing sugars for maintenance of growth and cellular metabolism under HT conditions. Earlier we also reported that in the developing grains of barley, HT induced invertase activities increased sink strength and alleviated the adverse effects of heat stress on assimilate partitioning (Singh *et al.* 2008). The observed increase in invertase activities was also related with increased RS contents (**Table 2**). Pressman *et al.* (2006) reported that heat stress condition altered the gene expression profiles of the enzymes, leading to an increased expression of AI in tomato anthers. This data indicated that sucrose-cleaving enzymes respond to HT conditions at both the mRNA and enzyme activity levels and may involve post-transcriptional control. By stimulating invertase activities, GA_3 established a more favourable sucrose gradient between the sink and source and played an important role in enhancing sink strength. Gibberellin-enhanced invertase activity has also been reported in etiolated pea seedling (Miyamoto *et al.* 1992) as well as in ovary protoplast (Estruch and Beltran 1991). ABA exerted its inhibitory effects either by directly affecting the enzymes activity or indirectly by limiting the stimulatory effect of GA_3. Chen and An (2006) also reported the antagonistic roles of GA_3 and ABA in barley.

Reduction in SS activities (**Table 1**) and hence sucrose synthesis at HT condition resulted in retention of reducing sugars in young growing embryonic axis (**Table 2**). Lafta and Lorenzen (1995) reported 59-72% reduction in SS activity in potato tubers in response to HT. Increased activities of both SS and invertases by GA_3 in HT stressed seedlings could result in higher turnover of sucrose, thereby countering the adverse effect of HT. In GA_3-treated pea internodes 70% higher SS activity was reported by Kordel and Kutschera (2000).

A significant increase in TS and RS contents in response to HS in root/shoot of treated seedlings was observed in both genotypes (**Table 2**). Reports in the literature indicated that increase in soluble sugars may be an adaptive mechanism for providing protection against HT stress and may protect cell against different stress factors (Bohnert *et al.* 1995; Huve *et al.* 2006). It may be possible that increased content of sugars some how activated the genes involved in stress tolerance. There is now compelling evidence that sugars are important mobile signals that regulate a variety of different genes. Sugars have also been reported to have different effects on various stages of plant development, which may be related to endogenous phytohormone levels (Yang *et al.* 2004; Gibson 2005).

These observed variations in the carbohydrate metabolism that occurred during HT stress could be very useful to understand the physiological events associated with seed germination and its development. The present study may form basis for understanding the effect of heat stress during seed germination and may provide initial platform for plant breeders to develop crop plants better adapted to high temperature conditions.

CONCLUSION

In the present study, GA_3 was found to play an important role in plant responses to HT condition. It seemed that GA_3 and invertases are effective tools to combat HT stress in barley and in future can be explored to promote the whole plant growth and final yield of the crop. Further detailed investigation of carbohydrate metabolism and hormonal interactions under heat stress conditions are needed to improve our understanding of the effect of HT on crop development.

REFERENCES

Acevedo E, Cardemil L (1997) Regulation of α-amylase isoenzyme expression in *Araucaria araucana* by gibberellic acid and abscisic acid. *Phytochemistry* **44**, 1401-1405

Amzallag GN, Lerner HR, Poljakoff-Mayber A (1992) Interaction between mineral nutrients, cytokinin and gibberellic acid during growth of sorghum at high salinity. *Journal of Experimental Botany* **43**, 81-87

Bohnert HJ, Nelson DE, Yansen RG (1995) Adaptations to environmental stress. *Plant Cell* **7**, 1099-1111

Chen K, An YQC (2006) Transcriptional responses to gibberellin and abscisic acid in barley aleurone. *Journal of Integrative Plant Biology* **48**, 591-612

Estruch JJ, Beltran JP (1991) Gibberellic acid stimulates acid invertase secretion in pea ovary protoplasts. *FEBS Letters* **279**, 303-306

Gibson S (2005) Control of plant development and gene expression by sugar signaling. *Current Opinion in Plant Biology* **8**, 93-102

Heikal MM, Shaddad MA, Ahmed AM (1982) Effect of water stress and gibberellic acid on germination of flax, seasame and onion seeds. *Biologia Plantarum* **24**, 124-129

Huve K, Bichele I, Tobias M, Niinemets U (2006) Heat sensitivity of photosynthetic electron transport varies during the day due to changes in sugars and osmotic potential. *Plant, Cell and Environment* **29**, 212-229

Kaplan F, Sung DY, Guy CL (2006) Roles of β-amylase and starch breakdown during temperatures stress. *Physiologia Plantarum* **126**, 120-128

Koc M, Barutcular C, Tiryakioglu M (2008) Possible heat-tolerant wheat cultivar improvement through the use of flag leaf gas exchange traits in a Mediterranean environment. *Journal of the Science of Food and Agriculture* **88**, 1638-1647

Kordel B, Kutschera U (2000) Effects of gibberellin on cellulose biosynthesis and membrane-associated sucrose synthase activity in pea internodes. *Plant Physiology* **156**, 570-573

Kumar J, Schaffer P, Huckelhoven R, Langen G, Baltruschat H, Stein E, Nagarajan S, Kogel KH (2002) *Bipolaris sorokiniana*, a cereal pathogen of global concern: cytological and molecular approaches towards better control. *Molecular Plant Pathology* **3**, 185-195

Lafta AM, Lorenzen JH (1995) Effect of high temperature on plant growth and carbohydrate metabolism in potato. *Plant Physiology* **109**, 637-643

Miyamoto K, Ueda J, Kamisaka S (1992) Sugar accumulation in growing subhooks in etiolated *Pisum sativum* seedlings. Stimulation of sugar exudations and invertase activity in epicotyls by gibberellic acid. *Physiolgia Plantarum* **84**, 106-111

Pagano EA, Benech-Arnold RL, Wawrzkiewicz M, Steinbach HS (1997) α-amylase activity in developing sorghum caryopses from sprouting resistant and susceptible varieties. The role of ABA and GA_3 on its regulation. *Annals of Botany* **79**, 13-17

Pagamas P, Nawata E (2008) Sensitive stages of fruit and seed development of chili pepper (*Capsicum annuum* L. var. Shishito) exposed to high-temperature stress. *Scientia Horticulturae* **117**, 21-25

Patric JW (1990) Sieve element unloading: cellular pathway, mechanism and control. *Physiolgia Plantarum* **78**, 298-208

Pressman E, Harel D, Zamski E, Shaked R, Althan L, Rosenfeld K, Firon N (2006) The effect of high temperatures on the expression and activity of sucrose-cleaving enzymes during tomato (*Lycopersicon esculentum*) anther development. *Journal of Horticultural Science and Biotechnology* **81**, 341-348

Russell E, Coupe S (Eds) (1994) *The Macqyarie World Atlas*, Macquarie Library Sydney, Sydney, Australia

Shinozaki K, Shinozaki YK (1996) Molecular responses to drought and cold stress. *Current Opinion in Biotechnology* **7**, 161-167

Singh R, Asthir B (1988) Import of sucrose and its transformation to starch in the developing sorghum caryopsis. *Physiologia Plantarum* **74**, 58-65

Singh S, Asthir B, Bains NS (2008) High temperature tolerance in relation to carbohydrate metabolism in barley. *Ecology, Environment and Conservation* **14**, 561-565

Sridevi V, Satyanarayana NV, Madhava Rao KV (1999) Induction of heat shock proteins and acquisition of thermotolerance in germinating pigeonpea seeds. *Biologia Plantarum* **42**, 589-597

Stommel JR (1992) Enzymic components of sucrose accumulation in the wild tomato species *Lycopersicon peruvianum*. *Plant Physiology* **99**, 324-328

Yang Z, Zhang L, Huang M, Wu N (2004) Sucrose regulates elongation of carrot somatic embryo radicals as a signal molecule. *Plant Molecular Biology* **54**, 441-459