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Correlated Changes of Some Amino Acids and Protease in Wheat Seedlings Subjected to Water and Temperature Stresses

By

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Summary

THIND S. K. & MALIK C. P. 1988. Correlated changes of some amino acids and protease in wheat seedlings subjected to water and temperature stresses. – Phyton (Austria) 28 (2): 261–269. – English with German summary.

Triticum aestivum L. cv. Sonalika seedlings subjected to water stress (−4 bars) and high temperature (45° C) shock (HTS) showed high accumulation of amides, cysteine and methionine, and decrease in hydroxylated amino acids. Additionally, enhancement of heterocyclic amino acids suggestively protects the cells from dehydration. Increased activity of protease following HTS indicated hydrolysis of proteins as a major contributing factor towards amino acid accumulation.

Zusammenfassung

THIND S. K. & MALIK C. P. 1988. Änderungen von Aminosäuren und Protease in Weizenkeimlingen nach Wasser- und Temperaturstress. – Phyton (Austria) 28 (2): 261–269. – Englisch mit deutscher Zusammenfassung.

Keimlinge von *Triticum aestivum* L. cv. Sonalika zeigten nach Wasserstreß (−4 bar) und Hochtemperaturschock (45° C) starke Akkumulation von Amiden, Cystein und Methionin und eine Abnahme hydroxylierter Aminosäuren. Zusätzlich läßt eine Zunahme heterozyklischer Aminosäuren eine Schutzwirkung gegenüber Austrocknung vermuten. Anstieg der Proteaseaktivität nach Hochtemperaturschock läßt Proteinhydrolyse als wesentlichste Ursache der Akkumulation von Aminosäuren erkennen.

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1. Introduction

Various metabolic parameters are advocated which could be successfully employed in recognising resistance of plants to water stress. One of the first reports that water stressed plants could accumulate proline was that of BARNET & NAYLOR 1966, who demonstrated 10–100 fold accumulation of this amino acid in stressed Bermuda grass (*Cynodon dactylon* [L.] PERS.). Subsequently SINGH & al. 1972, 1973 emphasized the accumulation of proline as an index of drought resistance, though later studies failed to confirm its universal acceptance (Reference given in SINGH & MALIK 1985). An overall enhancement in the total amino acids contents under water stress has also been demonstrated (DWIVEDI & al. 1979). HANSON & al. 1980 while evaluating the status of proline and betaine favoured latter as an index of drought resistance. Furthermore, the accumulation of proline is not a drought-specific response since it increased in plants subjected to low temperature (CHU & al. 1976) and salt stresses (CORHAM & al. 1986). In water-stressed plants, elevated levels of organic acids and carbohydrates (TIMPA & al. 1986) and protein (THAKUR & RAI 1980) have also been reported.

We have attempted to investigate the effect of water-stress and high temperature shock (45° C) for different time durations on the pattern of individual amino acids in Sonalika cultivar of wheat. Comparing metabolic status of plant under both stresses, the caus of amino acids accumulation is discussed.

2. Material and Methods

Plant material. Wheat (*Triticum aestivum* L.). cv. Sonalika recommended for cultivation in irrigated areas of Punjab was procured from the Department of Plant Breeding, PAU, Ludhiana. Of the various levels of water stresses (−2, −4, −6, −8 bars) tried, only −4 bars water stress was selected for the present studies. Water stress of −4 bars was induced by polyethylene glycol (PEG-6000) solutions (PARMAR & MOORE 1986). Fifteen seeds in four replicates were sown in sterile Petri-dishes lined with blotting paper containing PEG-6000 providing −4 bars osmotic stress or distilled water indicating zero stress as control. Free amino acids pattern was estimated in 3-day-old seedlings by paper chromatography. Experiment was repeated twice at 25±2° C in BOD (Biological Oxygen Demanding Incubator). For second set of experiment, seeds were germinated at optimum temperature (25±2° C) for 3 days with adequate supply of water. To study the effect of high temperature shock, these seedlings were transferred to high temperature (45° C) for 1, 2, 3 and 4 hr and change in total free amino acids was immediately estimated by the method of LEE & TAKAHASHI 1966.

Separation and estimation of free amino acids. To quantify individual amino acids, the material was homogenized with pestle and

mortar in presence of 80% ethanol (boiling) and centrifuged at 2000 × g for 10 min; the above step was repeated twice. This ethanol water soluble component was taken in separating funnel with 2 volumes of petroleum ether. After thorough shaking, ether layer was separated and the water-alcohol layer was washed.

The water-alcohol soluble material was further fractionated into sugars, amino acids and organic acids with cation exchange column (Dowex AG 50 W × 8, 200–400 mesh H^+ exchange) and anion exchange column (Dowex AG 1 × 8, 200–400 mesh formate column). Small ion exchange column (15–17 cm) was prepared from (7 mm diameter) corning glass tubing. The lower tip of column was plugged with fibre glass and filled with a slurry of resins (cleaned and loaded with H^+ or $HCCO^-$) in 80% ethanol to packed bed length of 4 cm. Prepared a tandem column by connecting the cation exchange column to the anion exchange column with a short piece of rubber tubing. Both the columns were neutralized by washing with 80% ethanol before assembly.

The water-alcohol fraction was slowly pipetted out on the top of the cation column, followed by slow elution of sugars (about one drop every

Table 1

Effect of moisture stress (−4 bars) on free amino acid (mg/g FW) spectra in 3-day-old seedlings of wheat cv. Sonalika

Amino acids	Control	−4 bars
Aliphatic amino acids		
Glycine	0.216	0.208
Alanine	0.081	0.324
Leucine and Isoleucine	0.200	0.486
Acidic amino acids (and their amides)		
Aspartic acid	0.513	0.108
Asparagine	0.297	0.351
Glutamine	0.243	0.297
Sulphur containing amino acids		
Cysteine	0.189	0.270
Methionine + Valine (Non-sulphur)	0.594	0.432
Hydroxylated amino acids		
Serine	0.243	0.243
Threonine	0.284	0.278
Heterocyclic amino acids		
Histidine	0	0.189
Tryptophan	0	0.122
Proline	0.180	0.640

Table 2

Effect of high temperature shocks (45° C) on free amino acid content (mg/g FW) in 3-day-old seedlings of Sonalika

Amino acids	Control (25 ± 2° C)	1 hr	2 hr	3 hr	4 hr at 45° C
Aliphatic amino acids					
Glycine	0.216	0.243	0.351	0.125	0.095
Alanine	0.081	0.648	0.673	1.026	0.284
Leucine and Isoleucine	0.200	0.440	0.484	0.360	0.459
Acidic amino acids (and their amides)					
Aspartic acid	0.513	0.397	0.337	0.312	0.108
Asparagine	0.297	0.297	0.314	0.351	0.162
Glutamine	0.243	0.453	0.513	0.621	0.243
Sulphur containing amino acids					
Cysteine	0.189	0.189	0.216	0.416	0.868
Methionine + Valine (Non-sulphur)	0.594	0.551	0.516	0.540	0.257
Hydroxylated amino acids					
Serine	0.243	0.243	0.257	0.284	0.283
Threonine	0.284	0.189	0.243	0.405	0.176
Heterocyclic amino acids					
Histidine	0	0.135	0.270	0.210	0.048
Tryptophan	0	0.189	0.162	0.162	0.027
Proline	0.180	0.321	0.422	0.651	0.821

10 sec) with 80% ethanol and collected 40 to 60 ml of neutral fraction (sugars) below the anion exchange column. The two columns were separated and amino acids were eluted from top column with 4 M NH_4OH in 80% ethanol. Ethanol extract was evaporated to dryness by heating on a water bath. The residue was taken in 3 ml of 20% ethanol and free amino acids were determined using method of YEMM & COCKING 1955.

Protease (E.C.3.4:4.1). For estimation of protease activity, method of BASHA & BEEVERS 1976 was followed.

3. Observations

3.1 Effect of water stress on pattern of free amino acids

The pattern of amino acids of water stressed (−4 bars) wheat seedlings was studied after 3 days of germination. The data on changes in individual amino acids contents are given in Table 1. Among the aliphatic amino acids,

leucine and isoleucine contents were more than double after 3 days of moisture stress. A four-fold increase in alanine was observed under stress though quantity of glycine was less than the control (zero stress). The quantity of acidic amino acid (aspartic acid) was lowered under stress, whereas that of its amide (asparagine) and glutamine increased. A general decrease was recorded under stress in hydroxylated amino acids (serine and threonine), whereas accumulation of sulphur containing amino acids (cysteine and methionine) and also valine enhanced. It is interesting to note that histidine and tryptophan (heterocyclic amino acids) which were not observed in the control accumulated under stress conditions. Proline though present under zero stress, increased six-fold when seedling was subjected to stress conditions.

3.2 Protease activity under water stress

Data in Table 3 represent protease level in the control and water stressed seedlings. Its specific activity in 3-day-old seedlings at −4 bars and −6 bars was more than the control.

Table 3

Effect of two levels of water stress on specific activity of protease (μ moles of tyrosine mg protein/hr) in 3-day-old wheat seedlings

Water potential (bars)	Specific activity	% increase over control
0 (Control)	1.81	–
− 4	1.93	+ 6.6
− 6	1.97	+ 8.7

3.3 Effect of high temperature shocks (45° C) on pattern of free amino acids

When 3-day-old seedlings were suddenly exposed to high temperature, the pattern of free amino acids altered (Table 2). There was accumulation of many individual amino acids along with proline which accumulated increasingly up to 4 hr of shock. The quantity of glycine, leucine and isoleucine (aliphatic amino acids) increased up to 2 hr and alanine up to 3 hr of shock (45° C) when compared with the control (25 ± 2° C), but declined with further increase in the incubation time at 45° C. Aspartic acid (acidic amino acid) decreased linearly with exposure to high temperature but its amide (asparagine) and glutamine, however, accumulated. Sulphur containing amino acids (cysteine and methionine) along with valine and serine (hydroxylated amino acids) did not increase at high temperature though threonine level enhanced four-fold. Additionally, accumulation of histidine, tryptophan along with proline (heterocyclic amino acids) was observed.

3.4 Protease activity as affected by high temperature shock

Specific activity of protease increased mani-fold when seedlings were exposed to high temperature up to 3 hr (Table 4).

Table 4

Protease activity in Sonalika cv. of wheat as affected by high temperature shock for variable intervals

Treatment periods (hr)	Specific activity	% increase over control
Control (25 ± 2° C)	1.81	–
1 hr at 45° C	7.50	314.36
2 hr at 45° C	8.50	369.61
3 hr at 45° C	5.79	219.88
4 hr at 45° C	2.48	37.01

4. Discussion

The role of amino acids and their amides as storage substances of nitrogen in plants is well documented and their accumulation under stress conditions has been established (GORHAM & al. 1986, RAI & al. 1983). Free proline accumulation has been reported frequently in plants subjected to water stress conditions (SINGH & al. 1972, 1973) and suggestively performs many functions, viz. a source of energy, stored component for nitrogen and carbon, keeps a cell in a turgid state and being protective in function (SINGH & MALIK 1985). The present studies reveal that along with proline other amino acids also accumulate which possibly help a plant to recover under stress. STEWART & al. 1977 have reported that proline accumulation was due to inhibition of its oxidation by water stress.

In Sonalika cultivar of wheat since very little increase (6.6%) was observed over control in protease activity, hydrolysis of proteins could not be the sole cause of large quantities of amides (asparagine and glutamine), which are important as structural parts of most proteins. Similarly, low level of protease has also been observed in susceptible cultivar of rice (MALI & METHA 1977). There is a possibility of synthesis of amides by other metabolic pathways, especially by amination of carboxylic acids by fixing ammonium nitrogen supplied from deamination of other amino acids liberated from proteins. Similar accumulation has also been reported in maize and cotton (THAKUR & RAI 1980, HANOWER & BRZOZOWSKA 1975). One of the attributes of dehydration is closure of stomata. Thus, metabolism under stress is retarded between central tetrapyrrole of chlorophyll affecting C fixation adversely. Contrarily, C fixed by PEP through PEP carboxylase produces oxaloacetic acid. Our studies have revealed that OAA was not used

to form malate (THIND 1986) but was utilized for the synthesis of alanine, leucine and valine (amino acids of pyruvate family). The desired N might be supplied by transamination from other amino acids, such as hydroxylated amino acids. Heterocyclic amino acids including proline, histidine and tryptophan were also accumulated. Histidine, one of the basic amino acids, is needed to produce histones. Accumulation of tryptophan might be due to decreased rate of its utilization to form auxin since under stress, seedlings were comparatively stunted in growth.

Seedlings exposed to high temperature shock (45° C) experienced sudden osmotic shock due to increased transpiration. The data given in Table 3 reveal that change in total free amino acids and pattern of individual amino acids was nearly similar to the seedlings exposed to water stress. Thus, proline accumulation increased with enhanced exposure time to high temperature (45° C). Similar effect of temperature has been reported earlier (CHU et al. 1976). We suggest that presence of large quantities of certain amino acids or their amides could be attributed to hydrolysis of proteins by protease. In a recent study, we reported that protein quantity in seedlings subjected to high temperature decreased initially and then increased due to the accumulation of specialized proteins (THIND & MALIK 1986). These were referred to as 'heat shock proteins' (HSP). According to MASCARENHAS 1984, when seedlings were suddenly exposed to high temperature, they adjust their metabolism in a way to prevent cells from dehydration. One such step is accumulation of free amino acids through shortest route of catabolism of proteins. After 3 or 4 hr period of heat shock, content of most individual amino acids which accumulated decreased. Concomitantly protease activity also decreased and this coincided with the time for maximum induction of HSP's (THIND & MALIK 1986). Protein degradation in leaves of *Lemna minor* transferred to adverse conditions has also been observed due to increase in activity of soluble proteolytic enzymes (protease). A model for stress induced protein degradation has been presented which envisages changes in membrane properties, especially of tonoplast causing vacuolar proteolytic enzymes leading to increased access to cytoplasmic proteins (COOKE et al. 1981).

Transamination of certain amino acids seems to cause accumulation of others, such as asparagine and glutamine – the structural proteins of most proteins, which act agents of N transport in plants. The reports on accumulation of thes amides under water stress are available (THAKUR & RAI 1980, HANOWER & BRZOZOWSKA 1975). In the present study, their accumulation under high temperature shock is shown for the first time. Apparently, under both water and high temperature stresses, wheat seedlings adapted themselves by altering their metabolism; accumulation of amino acids being one of them. Under water stress, accumulation is due to both synthetic and hydrolytic processes but with high temperature shock initially seedlings

adapt by hydrolyzing proteins and with time new specific proteins are synthesized.

5. Acknowledgements

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Recensiones

ELLENBERG H., MAYER R. & SCHAUERMANN J. (Hg.) 1986. Ökosystemforschung. Ergebnisse des Sollingprojekts 1966–1986. – Gr. –8°, 507 Seiten mit 233 Abbildungen und 145 Tabellen. Kunststoffeinband. – Verlag Eugen Ulmer, Stuttgart. – DM 120,–. – ISBN 3-8001-3431-4.

Im Rahmen des „Internationalen Biologischen Programms" (IBP) wurde in den Jahren 1966–1973 das erste globale Vorhaben auf dem Gebiete der Ökosystemforschung durchgeführt. Im Mittelpunkt des Projekts stand die Erforschung der Produktivität von terrestrischen, limnischen und marinen Ökosystemen. Als Rahmenthema wurde daher der Titel: „Biologische Grundlagen der Produktivität und der menschlichen Wohlfahrt" gewählt. Am Beitrag der Bundesrepublik Deutschland, dem bekannten, unter der Leitung von Prof. Dr. Heinz ELLENBERG stehenden Solling-Projekt, waren etwa 120 Wissenschafter verschiedenster Fachrichtungen beteiligt. Die Ergebnisse dieses fächerübergreifenden Forschungsprogramms wurden bereits in über 300 Einzelbeiträgen (vgl. dazu die Liste der Veröffentlichungen auf Seite 462–469) publiziert. Im vorliegenden Buchbeitrag geben die Mitarbeiter am Solling-Projekt einen Gesamtüberblick auch über jene Ergebnisse, die erst nach Abschluß des Schwerpunktprogramms hinzugekommen sind.

Als Untersuchungsflächen wurden vier im Hochsolling weitverbreitete Ökosysteme ausgewählt, und zwar ein saurer Hainsimsen-Buchenwald, der mit einem Siebenstern-Fichtenforst als Ersatzgesellschaft verglichen wurde; weiters wurden eine Rotschwingel-Goldhaferwiese bei unterschiedlicher Düngung und ein Weidelgras-Acker als kurzlebige Intensivkultur analysiert. In neun Abschnitten werden die Ergebnisse übersichtlich dargestellt und mit Tabellen und Abbildungen ausführlich dokumentiert. Im 1. Abschnitt werden, außer einer kurzen Definition des Ökosystembegriffes, die Ziele und Aufgaben des IBP und insbesondere des Solling-Projekts