General and Applied Plant Physiology – 2009, Volume 35 (1–2), pp. 88–92

Available online at www.bio21.bas.bg/ipp/

Short Communication

COMBINED EFFECT OF DROUGHT STRESS AND HEAT SHOCK ON CYCLOPHILIN PROTEIN EXPRESSION IN *TRITICUM AESTIVUM*

A. D. Sharma** and P. Kaur

Department of Biotechnology, Lyallpur Khalsa College, G T Road, Jallandhar-144001, Punjab, India

Received: *05 February 2009* Accepted: *15 June 2009*

Summary. The combined effect of drought stress and heat shock on the induction of cyclophilin proteins was studied in 3-day-old wheat seedlings. Imposition of stress resulted in a significant decrease in relative water content of shoots. Immunoblot analysis using polyclonal antibodies raised against a 20-kDa *A. thaliana* cyclophilin, revealed the induction of a cross-reacting band (45- kDa, wCyp-45), indicating its role in water stress adaptation. Based on these observations, the possible role of wCyp-45 in water stress tolerance is discussed.

Keywords*:* cyclophilin, drought, heat shock, wheat.

INTRODUCTION

Environmental stresses such as drought, salinity and heat significantly limit crop productivity. All these stresses are often interconnected and may induce similar cellular damage (Ingram and Bartels, 1996). In most of the studies on stress-associated proteins plants have been exposed to only one environmental stress factor - high temperature. Under field conditions, plants are often simultaneously exposed to soil drying and high temperature stresses. These two stress factors could create water deficit in plant tissues, which in turn may affect the synthesis of stress-induced proteins. Although abiotic stress response has been studied considerably in recent years (Chaves et al., 2003), however, analyzing the effect of a single stress factor on plants can be very different from conditions encountered by plants in the field in which a number of different stresses may occur simultaneously. These can alter plant metabolism in a novel manner that may be different from that caused by each of the different stresses applied individually. It may require a new type of response that would not have been induced by each of the individual stresses. Plants inherently possess various molecular and biochemical mechanisms that are involved in stress tolerance (Ingram and Bartels, 1996). One of these mechanisms that may confer stress tolerance is the activation of a large set of cyclophilins genes leading

*Corresponding author: arundevsharma@hotmail.com

to the accumulation of specific cellular proteins. Cyclophilins are the major group of stress-induced proteins believed to exert cellular protection during stress (Chou and Gasser, 1997). These proteins also seem to respond similarly to the application of ABA (Chaves et al., 2003; Kullertz et al., 1999). At present, hundreds of genes induced under drought stress have been identified which may allow plants to adapt to water limiting conditions. Because plant responses to environmental stresses are complex and multigenic, the functions of many of the induced genes are still a matter of conjuncture (Bray, 2002). Therefore, to better understand the role of these proteins in stress tolerance, it is a prerequisite to examine their expression under combined drought stress and heat shock. Thereafter, the sequencing of the relevant proteins and cloning of the corresponding genes will generate probes for early selection of drought resistant genotypes. Wheat is one of the most important crops in arid and semi arid areas worldwide and is sensitive to drought and temperature stress. In view of this, we chose wheat as an important tropical crop for the present investigation.

MATERIALS AND METHODS

Seed germination and growth conditions

The wheat seeds were surface sterilized with 1% (w/v) mercuric chloride followed by 70% (v/v) ethanol (Sharma et al., 2004). Seeds were thoroughly rinsed with deionized water and imbibed for 6 h. After imbibition, seeds were placed in Petri plates containing sterile filter sheets, moistened with water. The plates were incubated at 37±1°C in a seed germinator in darkness and allowed to grow for 3 days. For combined drought stress and heat shock treatment, 3-day-old seedlings were exposed to 2-d drought stress followed by 6-h heat shock (45°C). Individual drought stress was imposed to 3-day-old seedlings for 2 days. Heat treatment was imposed to 5-day-old seedlings for 6 h at 45°C. The tissues (shoots) from all treatments and control were harvested and pooled for further analysis. Relative water content (RWC) was measured after imposing stress conditions. Immediately tissues were sealed in a plastic bag and quickly transferred to the laboratory. Fresh weights were determined within 2 h after collection. Turgid weights were obtained after soaking leaves in distilled water in test tubes for 16 to 18 h at room temperature under low light conditions. After soaking, leaves were quickly and carefully blotted dry with tissue paper and turgid weight was determined. Dry weights were obtained after oven drying the samples for 72 h at 70°C. RWC was calculated according to the equation:

$$\text{RWC}(\%) = \text{fresh weight} - \text{dry weight} / \text{turgid weight} - \text{dry weight} \times 100$$

Extraction of proteins

Tissues (shoots) were homogenized with a chilled mortar and pestle in extraction buffer (50 mM Tris-HCl, pH 7.0). Crude extracts were centrifuged at 10,000×*g* for 10 min, and total protein content in the supernatant was determined according to the method of Bradford (Bradford, 1976). Proteins were resolved using SDS-PAGE on 15% (w/v) polyacrylamide gel and visualized by Coomassie brilliant blue as described by Sambrook et al. (1989).

Western blot analysis

Western blot analysis was carried out with antibodies (a gift from Dr C. S. Gasser)

raised against a 20 kDa *Arabidopsis thaliana* cyclophilin. After electrophoresis, proteins were electroblotted to a nitrocellulose membrane. Protein blots reacted with anti-Cyp20 (1:200 dilution) and developed using an alkaline phosphatase-conjugated secondary antibody (1 : 3000 dilution) and 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt/*p*-nitroblue tetrazolium chloride reagent systems (Sambrook et al., 1989).

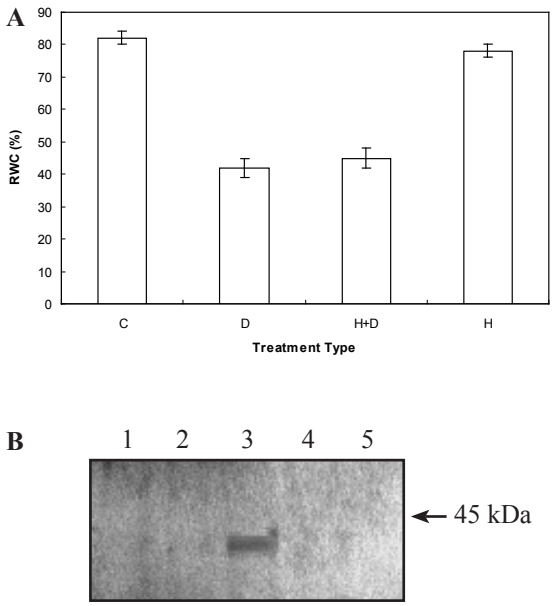

Figure 1 (A) Relative Water Content (RWC, %) of shoots under different stress treatments. Symbols: C – control; D – drought; H – heat; D+H – drought+heat. Data shown are means ± SE of three replicates. The letter "d" indicates significant differences vs. control at P≤0.05. (B) Immuno-blot analysis of wCyp-45 in *Triticum aestivum* after stress treatments. Each lane loaded with 60µg of total soluble proteins was resolved on 15% SDS-PAGE and transferred to a nitrocellulose membrane which was probed with antibodies against a 20-kDa cyclophilin of *A. thaliana*. Lane 1 - control; lane 2 - drought; lane 3 - drought+heat; lane 4 - heat; lane 5 - recovery (after combined treatments, seedlings were re-watered and kept at 37°C for 6 h).

Statistical analysis

A statview ANOVA program was used for statistical analysis of the data. Values for different treatments within each tissue were compared using one-way analysis of variance with repeated measures and Student's *t*-test for differences between pairs of data if the ANOVA ($LSD_{0.05}$) revealed significance. Means were tested by LSD at P≤0.05 level ($LSD_{0.05}$).

RESULTS AND DISCUSSION

In the present study, the effect of combined drought stress and heat shock on cyclophilin expression in wheat seedlings was studied. Our results strongly suggested that the effect of this combination on plants was very different from that of drought and heat shock applied individually. Because in the field conditions or in nature, plants are often subjected to a combination of stresses such as drought and heat shock, studying the response of plants to a combination of stresses may be critical to understand stress tolerance in plants. The response of plants to a combination of drought and heat shock resulted in a substantial decrease in the relative water content (RWC), indicating that seedlings were under stress. Similar decrease in RWC was also observed after individual drought stress (Fig. 1A). However, no significant change with respect to control was observed after heat shock.

Further, to gain insight into the molecular mechanisms of cyclophilin protein expression under combined drought and heat stress, western blot analysis was carried out using anti-Cyp-20 antibody. As a result we detected a strong protein band at about 45 kDa (wCyp-45) specifically under the combination of drought and heat shock (Fig. 1B, lane 3). In contrast, during heat shock and drought stress applied

individually, no cross reacting band was detected (Fig. 1B, lanes 1 and 2), suggesting that the combined stress affected plants differently from the individual stresses. It has been reported earlier for tobacco and maize that several HSPs or transcriptional factors such as a pathogenesis related factor (WRKY) and ethylene responsive transcriptional co-activator (ERCTCA), are induced or accumulate during drought stress and heat shock treatments which supports the presence of key regulators involved in this response (Ristic et al., 1991; Danyluk et al., 1991; Jacobsen and Shaw, 1989).

To gain further insight into the role of wCyP-45 in stress signaling in wheat seedlings, a post-stress study was carried out. Combined stress treatment (drought and heat shock) was removed by irrigating the seedlings with water followed by incubation at 37°C. Interestingly, the wCyp-45 band totally disappeared after removal of stresses (Fig. 1B, lane 5). Based on these observations it can be concluded that by virtue of the chaperonic and isomerase activity of cyclophilins (Boston et al., 1996), wCyp-45 may help other stress-induced proteins to mature besides regulating the expression of other genes imparting stress tolerance (Close et al., 1989). The specific induction of this protein under the combination of drought and heat shock may suggest that this combination is accompanied by the activation of a unique protein, which is not activated when each of these stresses was applied individually. Thus, it may be possible to enhance the tolerance of plants to multiple stresses by manipulating the expression of cyclophilins.

In conclusion, it was observed that wCyp-45 was specifically induced during combined stress treatment, suggesting that cyclophilins may be the potential candidates to be exploited in making various strategies of stress tolerance. Detailed studies with more drought tolerant and susceptible cultivars will reveal the potential of this protein as a marker for drought tolerance.

Acknowledgements: We are grateful to Prof. C. S. Gasser, Section of Molecular and Cellular Biology, Division of Biological Sciences, University of California, Davis, CA for the gift of *Arabidopsis thaliana* 20-kDa cyclophilin. This research was supported by the DST (Govt. of India) and management committee, Lyallpur Khalsa College, Jallandhar, Punjab, India.

REFERENCES

Boston, R.S., P.V. Viitanen, E. Vierling, 1996. Molecular chaperones and protein folding in plants. Plant Mol. Biol., 32, 191-222.

Bradford, M. M. 1976. A rapid and sensitive method for quantitation of microorganism quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72, 248-254.

Bray, E. A. 2002. Abscisic acid regulation of gene expression during water-deficit stress in the era of the Arabidopsis genome. Plant Cell Environ., 25, 153-161.

Chaves, M. M., J. P. Maroco, J. Pereira, 2003. Understanding plant responses to drought-from genes to the whole plant. Functional Plant Biol., 30, 239-264.

Chou, I. T., C. S. Gasser, 1997. Characterization of the cyclophilin gene family of *Arabidopsis thaliana* and phylogenetic analysis of known cyclophilin proteins. Plant Mol. Biol., 35, 873-892.

Close, T. J., K. A. A. Fenton, P.M. Chandler, 1989. A cDNA based comparisons of dehydration-induced proteins (dehydrins) in barley and corn. Plant Mol. Biol., 13, 95-108.

Danyluk, J., E. Rassart, F. Sarhan, 1991. Gene expression during cold and heat shock in wheat. Biochemistry Cell Biol., 69, 383-391.

Ingram, J., D. Bartels, 1996. The molecular basis of dehydration tolerance in plants. Annual Review Plant Physiol. Plant Mol. Biol., 47, 377-403.

Jacobsen, J.V., and D.C. Shaw, 1989. Heat-stable proteins and abscisic acid action in barley aleurone cells. Plant Physiol., 91, 1520-1526.

Kullertz, G., A. Liebau, A. Rucknagel, A. Schierhorn, B. Diettrich, G. Fischer, M. Luckner, 1999. Stress-induced expression of cyclophilins in proembryonic masses of *Digitalis lanata* does not protect against freezing/thawing stress. Planta, 208, 599-605.

Ristic, Z., D. J. Gifford, D.D. Cass, 1991. Heat shock proteins in two lines of *Zea mays* L. that differ in drought and heat resistance. Plant Physiol., 97, 1430-1434.

Sambrook, J., E. F. Fritsch, T. Maniatis, 1989. Molecular cloning: a laboratory manual, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York. USA. p 18.64-18.75.

Sharma, A. D., M. Thakur, M. Rana, K. Singh, 2004. Effect of plant growth hormones and abiotic stresses on germination, growth and phosphatase activities in *Sorghum bicolor* (L.) Moench seeds. African J. Biotechnol., 3, 308-312.