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The HPLC Profile of Proteins of Thermoprotection-Acquired Wheat (*Triticum aestivum* L.) Seedlings

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Abstract. A pre-treatment of 40 °C provided thermoprotection to wheat seedlings against 45 °C, which was otherwise a lethal temperature. Due to temperature pretreatment, the rate of protein synthesis at 45 °C increased in both plumules and radicles. The HPLC profile of plumule and radicle proteins of thermoprotection-acquired seedlings was different from the plumules and radicles of non-treated seedlings.

Additional index word : gel permeation HPLC.

At elevated temperatures, heat shock proteins of different molecular masses are synthesized in various plant species such as maize (Baszczynski *et al.* 1982), soybean (Lin *et al.* 1984), carrot, cotton, pea, sunflower and millet (Key *et al.* 1982) and pigeon pea (Kishore and Upadhyaya 1988). The exact function of heat shock proteins is still unknown, however, they are thought to protect cells against thermal damage. Sheoran *et al.* (1983) have developed a mutant WH 147M from a dwarf hexaploid wheat variety WH 147, which is reported to possess a higher thermal requirement. The seedlings of mutant WH 147M were also found to possess a more efficient thermoprotection system (Gupta *et al.* 1987). In this communication we report changes due to thermoprotection in proteins of plumules and radicles of WH 147 M and WH 147 wheat seedlings.

MATERIAL AND METHODS

Seeds of wheat cultivar WH 147M were obtained from the Department of Plant Breeding of this University. [^{3}H]-alanine was procured from the Isotope Division of Bhabha Atomic Research Centre, Trombay. Seeds were surface sterilized with 1% sodium hypochlorite and planted on moist filter paper at 30 °C.

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Temperature Treatment

Two day old germinated seeds were given the desired temperature treatment in a medium containing 50 mM phosphate (pH 6.0), 30 mM sucrose and 0.15 mM chloramphenicol in a shaking water bath. After the temperature treatment the seedlings were washed and replanted on a moist filter paper and kept at 30 °C, in light.

Incorporation of [^{3}H]-Alanine

For the measurement of protein synthesis, plumules and radicles 10 each were excised from two day old germinated seeds and incubated for 4 h in 1.5 ml 50 mM phosphate (pH 6.0), 30 mM sucrose and 0.15 mM chloramphenicol at the desired temperature in a shaking water bath. [^{3}H]-alanine ($3MB_2$; 80 μli) was added to each test tube, during the final 2 h period of incubation. The incubation was stopped by chilling and plumules and radicles were washed with 2 mM alanine and chilled water. Proteins were extracted in 60 mM Tris (pH 6.8) containing 0.6 M 2-mercaptoethanol and 30 mM sodium dodecyl sulfate. The crude homogenate was centrifuged at 12 000 × *g* for 15 min and the supernatant was used for radioactivity measurement. The filter paper disk method of Mans and Novelli (1961) was used for counting 10 % trichloroacetic acid insoluble radioactivity by Beckman Liquid Scintillation counter. Proteins were estimated by the method of Lowry *et al.* (1951).

High Performance Liquid Chromatography

Protein from the plumules and radicles were extracted in 50 mM phosphate (pH 6.5) containing 0.3 M NaCl and 0.2 mM sodium azide. The homogenate was centrifuged at 12 000 × *g* for 15 min and the supernatant was passed through 0.4 μm porosity filter. Shimadzu model LC 4A fitted with HSG-30W gel permeation column was used for the chromatography. The proteins were eluted by 50 mM phosphate (pH 6.5) containing 0.3 M NaCl and 0.2 mM sodium azide at a flow rate of 1 ml min^{-1}. The elution profile of proteins was detected using SPD-2AS detector and recorded by CR 2AX data system. The elution profile of replicated samples was identical.

RESULTS

Two days old wheat seedlings, having received a 45 °C heat shock for 2 h, when returned to normal temperature (30 °C) showed no subsequent plumule and radicle growth (Fig. 1). However, a pre-treatment of 40 °C for 2 h provided protection of growth against 45 °C heat shock. The growth of radicles was more affected by heat shock and thermoprotection by the pre-treatment was also relatively less in radicles

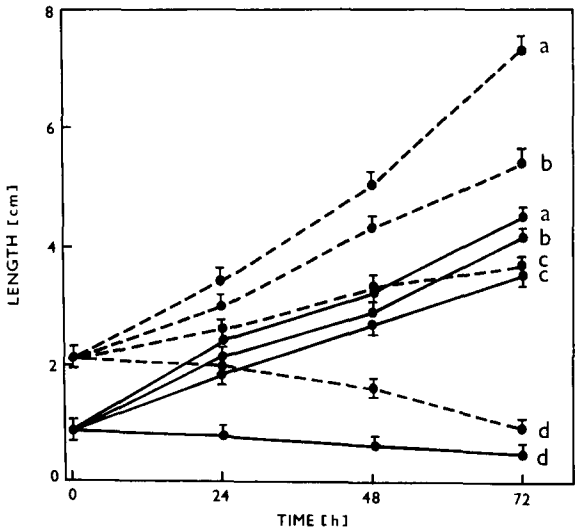

Fig. 1. Effect of temperature on the growth of 2-day-old-wheat seedlings. Seedlings, incubated at 30 °C, 4 h (a); 30 °C, 2 h → 40 °C, 2 h (b); 40 °C, 2 h → 45 °C, 2 h (c); 30 °C, 2 h → 45 °C, 2 h (d); were transferred to 30 °C and plumule (full line) and radicle (dashed line) growth was measured.

as compared to plumules. The incorporation of labelled amino acid into proteins extracted from plumules and radicles which were subjected to temperature treatment is shown in Table 1. The rate of label incorporation decreased to 25 per cent of the control value in both plumules and radicles during 45 °C heat shock. A treatment of 40 °C for 2 h to plumules and radicles resulted in higher levels of protein synthesis at 45 °C. Proteins from plumules and radicles of seedlings received different

TABLE 1

Effect of temperature on the incorporation of ^{3}H-alanine in plumules and radicles of 2-day old wheat seedlings

Treatment	[^{3}H]-alanine incorporation [cpm mg^{-1} (protein)]	
	Plumule	Radicle
30 °C, 4 h	9279 ± 571	48651 ± 477
20 °C, 2 h → 40 °C, 2 h	6681 ± 587	19603 ± 798
30 °C, 2 h → 45 °C, 2 h	2145 ± 210	11998 ± 497
40 °C, 2 h → 45 °C, 2 h	3784 ± 130	20496 ± 359

Mean ± s.d.

temperature treatments and were separated by gel permeation HPLC. The HPLC pattern of plumule and radicle proteins is shown in Fig. 2. Proteins of plumules of control seedlings resolved into 15 components, white those of seedlings which received 40 °C treatment for 2 h followed by 45 °C treatment for 2 h showed 18 components. Comparison of HPLC profiles of temperature pre-treated and 45 °C exposed plumules with control plumules showed that 12 components were common, 6 were new and 3 were lost components. The number of components in control and temperature treated radicles were 13 and 11 respectively. The comparison of HPLC profiles of temperature treated with control radicles showed that 8 components were common, 3 were new and 5 were lost. The size of different proteins which appeared anew during heat shock ranged between 110 and 18 kD. Two of these, 110 and 30 kD were common to both the plumules and radicles. In plumules the size of the other proteins which appeared during heat shock was 85, 55, 47 and 18 kD.

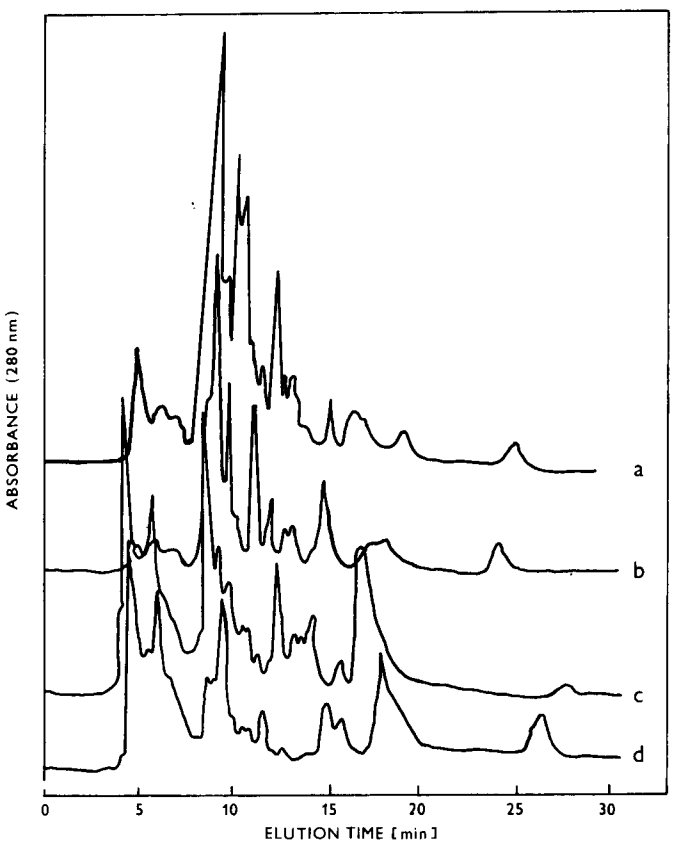

Fig. 2. The HPLC profiles of proteins of radicles and plumules of 2-day-old wheat seedlings incubated at 30 °C, 4 h (b-plumule, d-radicle) and 40 °C, 2 h → 45 °C, 2 h (a-plumule, c-radicle).

DISCUSSION

Several plant species and cells in culture are known to possess a temperature inducible system of heat shock protection mechanism (Atkinson and Walden 1985). The response of 2 day old thermoinduced wheat plumules and radicles to 45 °C was somewhat analogous to soybean (Lin *et al.* 1984) and maize (Cooper and Ho 1983). Thermal protection is reported to be due to the production of some proteins termed as heat shock proteins. The rate of insorporation of labelled amino acid into proteins of plumule and radicles indicated that a heat shock of 45 °C, reduced protein synthesis drastically. In maize plumules, Baszczynski *et al.* (1982) reported a similar reduction in protein synthesis above 41 °C. The reduction in protein synthesis seedlings at elevated temperatures was reported to be due to a rapid conversion of polyribosomes into monoribosomes (KEY *et al.* 1981). Induction of heat shock protection mechanism is accompanied by protein synthesis. In the present study a prior treatment of 40 °C for 2 h enhanced the rate of protein synthesis at 45 °C. A wide range of organisms including bacteria (Patterson and Gillespie 1971), lower eukaryotes (Francis and Lin 1980), insects (Ashburner and Bonner 1979) and plants such as soybean (Lin *et al.* 1984), maize (Baszczynski *et al.* 1982) and tobacco culture cells (Meyer and Chartier 1983) respond to elevated temperature by synthesizing a new set of proteins.

Proteins and polypeptides produced during heat shock have been detected in several organisms including plants mainly by fluorography of labelled proteins separated by electrophoresis. In general, analysis of heat shock shows that some proteins synthesized at normal temperature continue to be synthesized at elevated temperature. A second group of proteins is of those, whose synthesis is inhibited and a third group includes newly synthesized heat shock proteins which are undetectable at normal growth temperature (Key *et al.* 1981). In the present work, radicle and plumule proteins were separated by HPLC and the results obtained were in general similar to those reported by electrophoretic analysis in other plant species (Barnett *et al.* 1980, Key *et al.* 1981, Baszczynski *et al.* 1982, Cooper and Ho 1983, Kishore and Upadhyaya 1988). The exact function of the plant heat shock proteins still remains to be identified. Since HPLC technique separates native proteins, the 'stress proteins' identified by this technique may facilitate their functional analysis.

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BOOK REVIEW

Marten, G. C. (ed.): Grazing Research: Design, Methodology, and Analysis. CSSA Special Publication Number 16. – CSSA, SSSA and ASA, Madison 1989. 136 pp. Hardcover US $ 20.–.

This special publication encompasses the papers from a symposium held at the annual meeting of the Crop Science Society of America in Anaheim, CA, in November 1988.

The publication consists of 10 papers which describe methods of establishing grazing trials, their optimum design and a way of management. Necessity of judicious planning and clearly stated objectives of the study is accentuated to obtain a better explanatory power of the results provided that economic considerations in grazing research are respected. Statistic methods of the evaluation of grazing trials and ways of reducing sampling cost or experimental errors are discussed. It is necessary to coordinate efforts of researchers and statisticians to enhance the standard of the experiments.

The volume complemented by references will be useful mainly to grazing researchers and all specialists interested in the plant-animal interface.

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