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Early and Late Heat Shock Proteins in Wheats and Other Cereal Species[1]

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ABSTRACT

Coleoptiles and roots of 3-day-old seedlings from five cereal species (*Triticum aestivum* L., *T. durum* Desf., *Hordeum vulgare* L., *Secale cereale* L., and *Triticale*) respond to heat shock at 40°C by synthesizing a new set of 13 strong bands (as revealed by one-dimensional sodium dodecyl sulfate gel electrophoresis) as well as some 20°C proteins. Heat shock proteins (HSPs) belong to three different size groups: high molecular mass HSPs in the 103 to 70 kilodalton range, intermediate molecular mass HSPs in the 62 to 32 kilodalton range, and low molecular mass HSPs about 17 to 16 kilodalton in size. At the beginning of the heat shock coleoptiles show a reduced ability to synthesize intermediate molecular mass HSPs but after 4 hours at 40°C they exhibit fully developed HSP patterns identical to that found in roots. Synthesis of early HSPs declines after 7 hours of treatment followed by the appearance of a new set of 12 protein bands (late HSPs) in the ranges 99 to 83, 69 to 35, and 15 to 14 kilodaltons. After 12 hours at 40°C, three other late HSPs of 89, 45, and 38 kilodalton are induced. The induction of late HSPs after 7 hours at 40°C appears to be associated with an enhancement of radioactive methionine incorporation into proteins.

It has been shown that several species of higher plants including soybean, pea, tobacco, tomato, cotton, sorghum, and maize respond to high temperature (39–45°C) stress by synthesizing HSPs[2] which are undetectable at normal growing temperature (see Ref. 16 for a review). Kinetics of HSP synthesis (10), their selective localization with cell structures (1, 8), and accumulation in leaves or chlorenchima of temperature-stressed mature plants (4, 7, 9) suggest that HSPs could provide the basis of thermoprotection. The isolation of mutants deficient in the expression of HSPs and unable to develop thermotolerance (11, 13) as well as the induction of HSP-like proteins by the respiratory inhibitor arsenite with a concomitant development of thermal tolerance (10) are two lines of evidence for a role of HSPs in providing cellular defence against high temperature.

In cereal species, studies on HSPs have been carried out in maize (2, 5, 6) and, to a lesser extent, sorghum (15) and barley (12). In maize, the induction of HSPs occurs in two phases: the immediate synthesis of 10 HSPs (as revealed by one-dimensional SDS gel electrophoresis) and then the appearance of three new polypeptides after a prolonged high temperature treatment (5). Moreover, all maize tissues exhibit a similar set of HSPs with some tissue-specific differences in the electrophoretic pattern (6).

Here we report on the heat-shock response of roots and coleoptiles in several varieties of both common and durum wheat as well as in other cereal species like barley, rye, and triticale.

MATERIALS AND METHODS

Plant Materials. Cultivars or lines of common wheat (*Triticum aestivum* L., cv Salmone, Manital, S. Pastore, Orso, Livio, Chinese Spring, and Chejenne), two cultivars of durum wheat (*T. durum* Desf., cv Creso and Karel), one cultivar of barley (*Hordeum vulgare* L., cv Onice), rye (*Secale cereale* L. cv Cinquecento), and triticale (*T. aestivum* × *S. cereale*, cv Gloria) were used in these experiments.

***In Vivo* Labeling and Protein Extraction.** Seeds sterilized for 5 min in 5% NaOCl and rinsed thoroughly with distilled water were germinated at 20°C in the dark in Petri dishes upon filter paper kept moist with water. Six 3-d-old seedlings (with endosperms removed) were transferred to a small tube containing 1 ml of incubation buffer (2% sucrose in 10 mM K-phosphate buffer, pH 6.5) in a shaking water bath at 20°C or 40°C (±1°C). After 1 h, 50 μCi/ml of [^{35}S]methionine (>1000 Ci/mmol, obtained from Amersham) were added and the seedlings were incubated for 2.5 to 3 h. In some experiments, seedlings were kept at 40°C for 2, 4, 6, or 12 h before labeling with [^{35}S] methionine for 2.5 to 3 h. Labeled seedlings were rinsed with nonradioactive methionine and their coleoptiles and roots were excised. Either two coleoptiles or six roots were transferred to an Eppendorf centrifuge tube and ground under liquid N_2; the powder was resuspended in 250 μl of cold extraction buffer (5% SDS, 5% 2-mercaptoethanol in 200 mM Tris-HCl, pH 7.5). After 30 min at 0 to 4°C the homogenate was boiled for 3 min and centrifuged for 30 min in an Eppendorf microfuge. Aliquots of the supernatant were dissolved in Insta-gel scintillation cocktail (Packard, USA) and counted to estimate total uptake of [^{35}S] methionine. Incorporation of [^{35}S]methionine into proteins was determined by 10% TCA precipitation; the insoluble material was filtered on nitrocellulose filter and counted.

Gel Electrophoresis of Proteins and Fluorography. Immediately before use 35 μl of supernatant prepared as described above were mixed with 15 μl of a sample buffer containing 30% (v/v) glycerol, 6% SDS, 15% 2-mercaptoethanol, 0.003% pyronin Y in 0.2 M Tris HCl (pH 6.8). Nearly the same number of cpm (30,000) was loaded into each sample well of a 3% (w/v) polyacrylamide stacking gel on top of a polyacrylamide separating gel containing 10, 15, or 20% acrylamide (see figure legends for specific acrylamide concentrations). The following mol wt markers were used: Cyt *C* (12.3 kD), lactoglobulin A (18 kD), carbonic anydrase (30 kD), ovalbumin (46 kD), albumin (69 kD), phosphorylase B (97.4 kD) as [^{14}C]methylated form. Gels were prepared for fluorography as described by Bonner and Laskey (3), dried, and exposed to Kodak X-Omat AR film at −80°C.

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[2] Abbreviation: HSPs, heat shock proteins.

RESULTS

Effects of Heat Shock on the Protein Pattern. When seedlings of durum wheat are transferred to 40°C and labeled for 3 h after 1 h preincubation at this temperature the pattern of protein synthesis changes significantly and new bands undetectable at 20°C are produced (Fig. 1). Three different size groups of HSPs (indicated by bars) are synthesized in heat shocked roots (Fig. 1, lane B): a group of 5 high molecular mass HSPs (103–70 kD in size), a group of 5 intermediate mol wt HSPs (59–32 kD in size), and a small group of 2 low molecular mass HSPs (about 17–16 kD in size). Besides these 12 HSPs other minor HSPs can be seen as faint bands on the autoradiogram of heat-shocked roots. In coleoptiles (Fig. 1, lane C) the 40°C treatment induces a similar pattern of HSP synthesis; however, many intermediate mol wt HSPs are synthesized in lower amounts. Figure 2 shows that the HSP patterns in common wheat cultivars are identical to those synthesized in durum wheat.

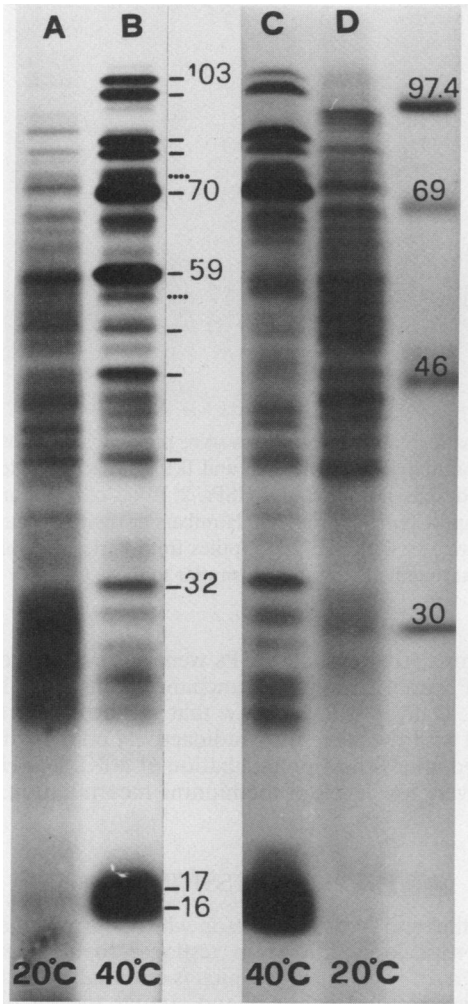


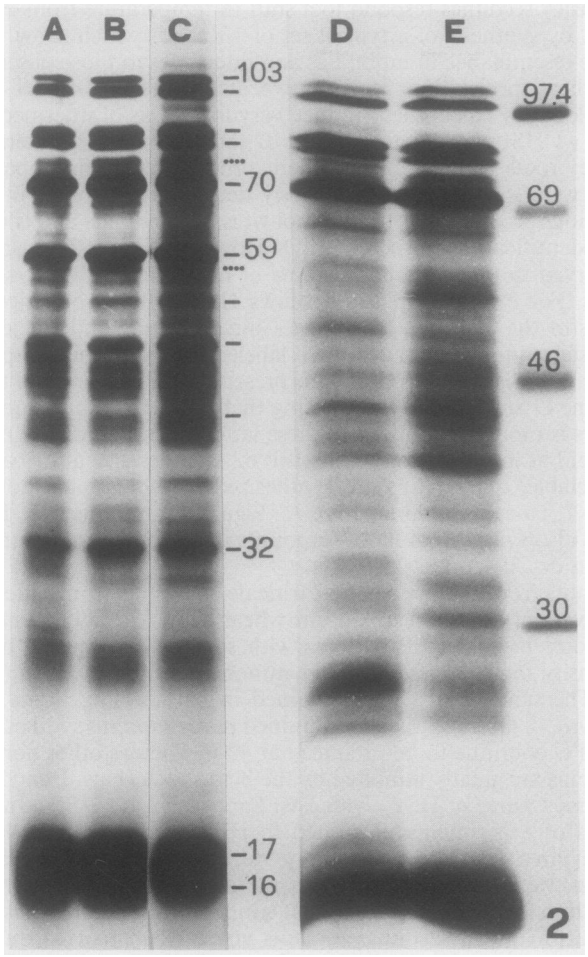
FIG. 1. Pattern of protein synthesis in roots (lanes A and B) and coleoptiles (lanes C and D) of durum wheat cv Karel subjected to temperatures indicated below each line. Seedlings were incubated for 4 h at 20 or 40°C and labeled with [^{35}S]methionine in the final 3 h of the experiment. Protein were separated on a SDS gel containing 15% acrylamide and 0.1% bis-acrylamide. Bars and associated numbers indicate major HSPs and the molecular mass in kD; dotted lines indicate minor HSPs. Molecular mass (kD) of protein standards are reported in the right margin.

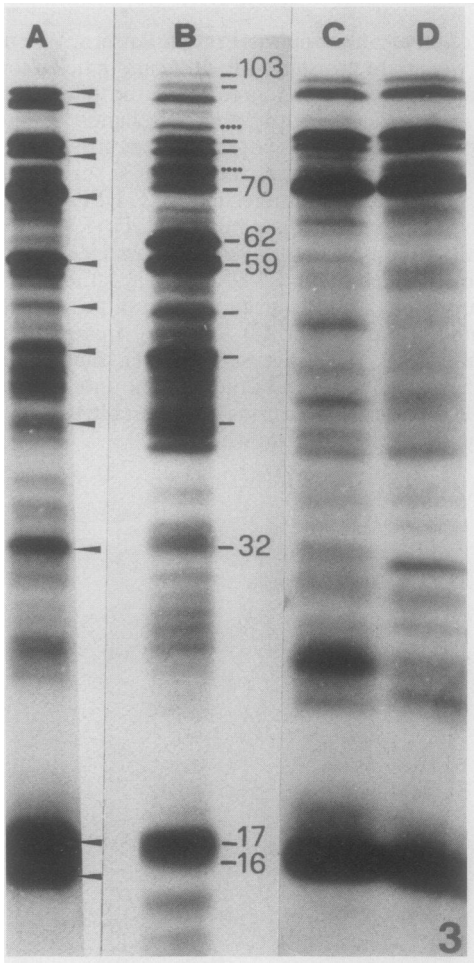
Barley seedlings respond to a shift in temperature from 20 to 40°C by synthesis of a typical set of 13 bands which show very close resemblance in mobilities and intensities to those observed in wheat species (Fig. 3). As in wheats, in the first 4 h of heat shock barley coleoptiles produce very low levels of intermediate mol wt HSPs. As Figure 3, lane B shows, the HSP pattern in barley roots contains a prominent protein having an apparent mol wt of 62,000, which is barely detectable or absent in the previous protein patterns (the mol wt is approximate and is used for identification purposes only). However, HSP 62 has been observed in other autoradiograms of heat-shocked wheat seedlings. For example, Figure 4 shows HSP patterns obtained in roots of the common wheat cvs Chinese Spring and Chejenne transferred to 40°C for 1 h, then labeled for 3 h at this temperature. In lanes B and C HSP 62 is present as a stronger band than in lane D and E; it is worth noting that synthesis of high mol wt HSPs is more pronounced in these latter lanes where HSP 62 is present at lower levels. When HSP 62 is either absent or barely detectable, other HSPs are synthesized in increasing amounts (Fig. 1, lane B; Fig. 3 lane A). As Figure 4, lane F shows, HSP 62 is also synthesized in low amounts in coleoptiles of common wheat cv Chejenne.

Roots from barley, rye, and triticale as well as from different cultivars of common or durum wheat show virtually identical one-dimensional HSP patterns with some differences in band intensity for HSP 62 and other minor high mol wt HSPs (Fig. 5). The same results were obtained in oat seedlings (data not shown). In all cereal species examined many proteins synthesized at 20°C continue to be produced at 40°C whereas other normal proteins are greatly inhibited by the heat shock (Figs. 1 and 4).

Time Course of HSP Synthesis: Early and Late HSPs. In the series of experiments illustrated in Figures 6 and 7, seedlings from three cereal species (barley, durum wheat, and common wheat) were transferred to 40°C and labeled for 3 h after preincubation for 1, 2, 6, or 12 h at that temperature. The HSP pattern in durum wheat seedlings remains virtually unchanged during the first 5 h of heat shock (Fig. 6a, lanes B, C, F, and G); after 6 h of preincubation, synthesis of the 'early', original 12 HSPs declines and 12 'late' HSP bands begin to be synthesized (Fig. 6a, lanes D and H). As with the early HSPs, these new proteins belong to three size groups in the ranges 99 to 83 kD (high mol wt HSPs), 69 to 35 kD (intermediate mol wt HSPs), and 15 to 14 kD (low mol wt HSPs) and are accompanied by other minor bands similar in mobility to bands present at the control temperature. Common wheat or barley seedlings show the same time course of protein synthetic response to heat shock, exhibiting the typical set of 12 late HSPs (Figs. 6b and 7). Moreover, when barley seedlings are maintained at 40°C for 12 h before labeling, there is an induction of an additional group of three HSPs of 89, 45, and 38 kD (Fig. 7, lanes D and G) whereas synthesis of late HSP 63 and HSP 64 becomes maximal. It would be interesting to check whether these 3 HSPs are also synthesized in the other cereal species. Additionally, from experiments with barley roots it appears that synthesis of HSP 62 which appears as a strong band after 1 h of preincubation at 40°C (Fig. 7, lane A), is transient and declines upon prolonged heat shock treatments (lane B and C). Figure 8 shows the HSP patterns of seedlings from several common wheat lines transferred to 40°C for 4 h, then labeled for 3 h at that temperature. The observation that synthesis of early HSPs is more pronounced whereas late HSPs are not yet visible would indicate that induction of late HSPs occurs after a global period of 7 h of heat shock. Most notable is the increased intensity of intermediate mol wt HSPs in coleoptiles whose HSP patterns after prolonged heat stress are indistinguishable from those obtained in roots.

When experiments of prolonged treatments at 40°C were carried out on other cultivars or lines of both common and durum wheat, late HSPs were not induced in some and early HSPs





FIGS. 2 and 3. Protein patterns from roots and coleoptiles of common wheat or barley cultivars. Seedlings were preincubated at 40°C for 1 h then labeled for 3 h at this temperature. Proteins were separated on SDS gels containing 15% acrylamide and 0.1% bis-acrylamide. Bars and associated numbers indicate major HSPs and the molecular mass in kD whereas dotted lines indicate minor HSPs. 2: Roots of common wheat cvs Salmone (lane A), Chinese Spring (B), Chejenne (C); coleoptiles of cvs Salmone (D) and Chinese Spring (E). Numbers at right indicate mol wt of protein standards. 3: Roots from common wheat cv Chinese Spring (lane A) and barley cv Onice (B); coleoptiles from barley cv Onice (C) and common wheat cv Chinese Spring (D). Arrow heads indicate common wheat HSPs that migrated with mol wt similar to those of barley HSPs.

continued to be synthesized throughout the heat shock. This aspect will be considered later.

Effect of Heat Shock on the Uptake and Incorporation of Methionine. The uptake of [^{35}S]methionine is affected by the transfer of seedlings from 20 to 40°C in all three cereal species shown in Figure 9 (uptake). After 6 h of preincubation at 40°C the uptake is reduced up to 25% of the control level. The incorporation of labeled amino acid into polypeptides undergoes a marked reduction after transfer to 40°C. As incorporation is affected by the greatly reduced uptake of radioactive methionine, the effect of heat shock on the rate of protein synthesis was measured as the percentage radioactivity bound with the TCA-precipitable material (Fig. 9, incorporation). All three cereals exhibit a substantial drop in protein synthesis after 1–2 h of preincubation at 40°C followed by an increase and stimulation after 6 h. Protein synthesis falls back to a very low level after 12 h of preincubation.

Association between Late HSPs and Rate of Incorporation. When measurements of incorporation were performed in other cultivars or lines of both common and durum wheat, stimulation of protein synthesis after 6 h of preincubation at 40°C was not observed in some and the percentage radioactivity in proteins decreased continuously with the increase of preincubation period. In these seedlings late HSPs were not synthesized and the early HSP pattern remained unchanged during 9 h of 40°C treatment. Data in Table I show that synthesis of late HSPs is associated with the percentage radioactivity bound with proteins synthesized after 6 h of preincubation at 40°C, late HSPs being absent at very low levels of methionine incorporation.

DISCUSSION

Coleoptiles and roots of common wheat, durum wheat, barley, rye, and triticale synthesize in response to high temperature treatment a set of early HSPs which is essentially the same in the different species and cultivars and similar to that observed in maize tissue (5, 6). At the beginning of heat shock coleoptiles appear to synthesize lower levels of intermediate mol wt HSPs in comparison with roots but, if the 40°C treatment is prolonged, they show HSP patterns indistinguishable from those in roots. Although the physiological significance of this phenomenon is not yet clear, it has been suggested that coleoptiles could be less sensitive to high temperature than roots (12).

Synthesis of HSPs is actually multiphasic, induction of early HSPs followed by synthesis of two successive sets of late HSPs.

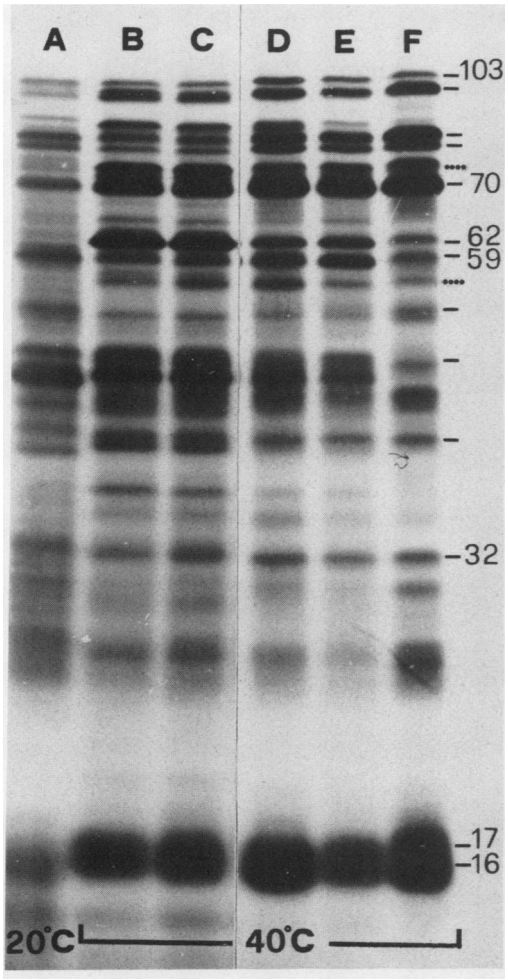


FIG. 4. Patterns of proteins synthesized by roots or coleoptiles of common wheat cultivars Chinese Spring (lanes A, B, and D), and Chejenne (lanes C, E, and F) at 20°C or after a heat shock at 40°C. Labeling of seedlings was carried out for 3 h at 40°C after 1 h of preincubation at this temperature. SDS gel contained 15% acrylamide and 0.1% bis-acrylamide. Bars and associated numbers at right indicate major HSPs and the molecular mass in kD whereas dotted lines indicate minor HSPs. Lanes: A, roots at 20°C; B, C, D, and E, roots at 40°C; F, coleoptiles at 40°C.

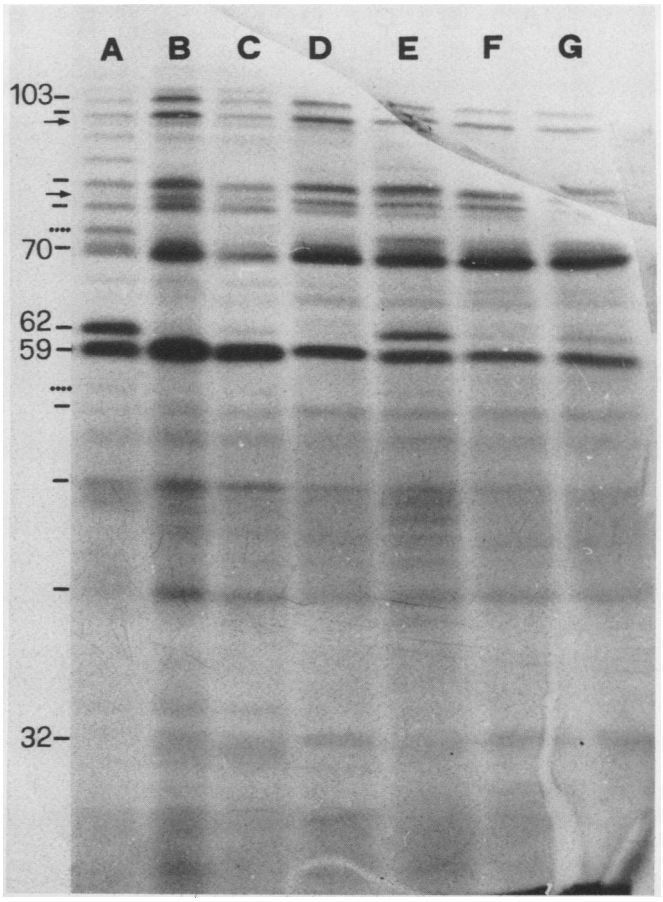


FIG. 5. Autoradiograms of proteins synthesized by heat-shocked roots of barley cv Onice (A), rye cv Cinquecento (B), common wheat cvs Livio (C), Orso (F), and S. Pastore (G), durum wheat cv Karel (D), and triticale cv Gloria (E). Seedlings were labeled for 3 h at 40°C after 1 h of preincubation at this temperature. Proteins were separated on a SDS gel, containing 10% acrylamide and 0.13% bis-acrylamide. Bars and associated numbers at the left margin indicate major HSPs and the molecular mass in kD whereas dotted lines indicate minor HSPs. Arrows indicate minor HSPs not separated in more concentrated gels. Low mol wt HSPs are not present in these autoradiograms because they had eluted from the bottom of the gel.

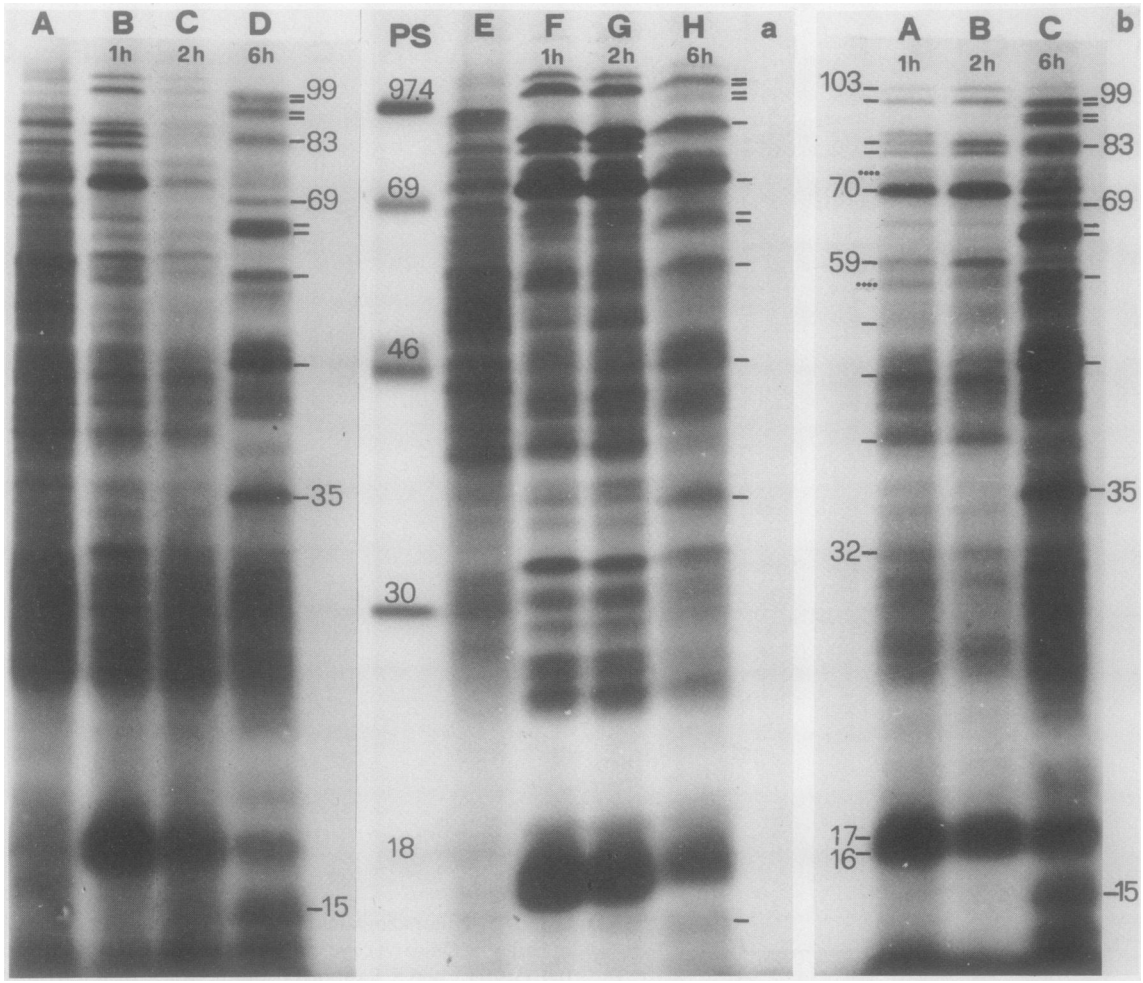


FIG. 6. Time course of protein synthesis in roots or coleoptiles of durum wheat cv Karel (a) and common wheat cv Salmone (b). Seedlings were labeled for 3 h at 40°C after preincubation for increasing lengths of time at this temperature. Control seedlings were incubated and labeled at 20°C. Proteins were separated on a SDS gel containing 15% acrylamide and 0.1% bis-acrylamide. a: roots at 20°C (A); roots at 40°C for 1 h (B), 2 h (C), and 6 h (D) preincubation; coleoptiles at 20°C (E); coleoptiles at 40°C for 1 h (F), 2 h (G), and 6 h (H) preincubation. b: roots at 40°C for 1 h (A), 2 h (B), and 6 h (C) preincubation. Bars and associated numbers at right of lanes D (a) and C (b) indicate major, 'late' HSPs and the molecular mass in kD. Bars and associated number at the left margin in (b) indicate major, early HSPs and the molecular mass in kD whereas dotted lines indicate minor, early HSPs. Mol wt of protein standards (PS) are reported in (a).

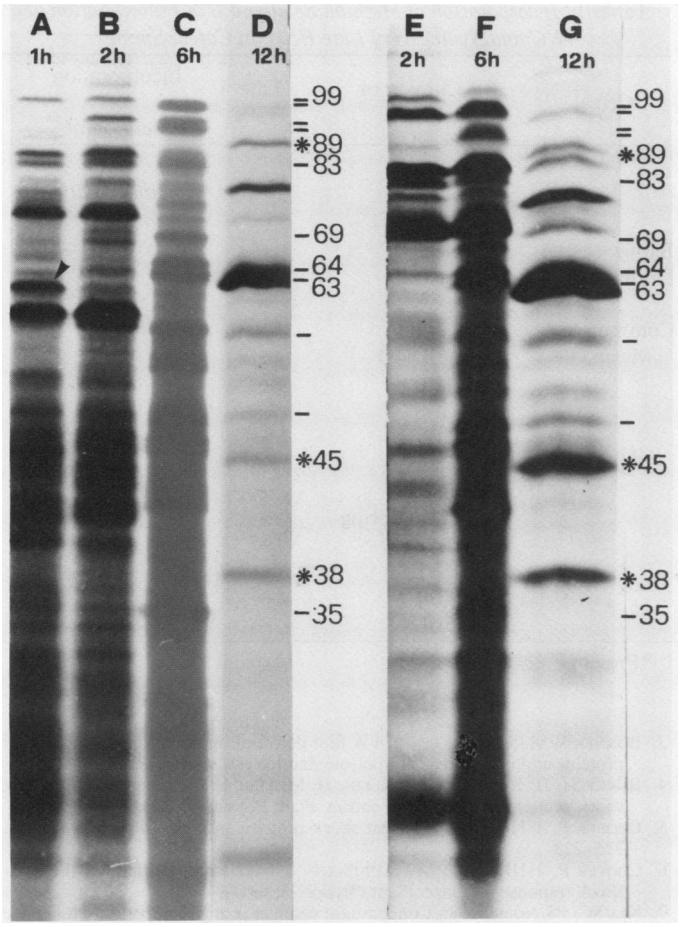


FIG. 7. Time course of protein synthesis in roots (lanes A, B, C, and D) or coleoptiles (lanes E, F, G) of barley cv Onice. Seedlings were labeled for 3 h at 40°C after preincubation for increasing lengths of time at this temperature. The SDS gel contained 15% acrylamide and 0.1% bis-acrylamide. Lanes: preincubation for 1 h (A), 2 h (B and E), 6 h (C and F), 12 h (D and G). Bars and associated numbers indicate major late HSPs and the molecular mass in kD. Asterisks denote late HSPs synthesized after 12 h preincubation at 40°C. Arrow head indicates HSP 62. Low mol wt HSPs (early and late) are not present in the autoradiograms because they had eluted from the bottom of the gel.

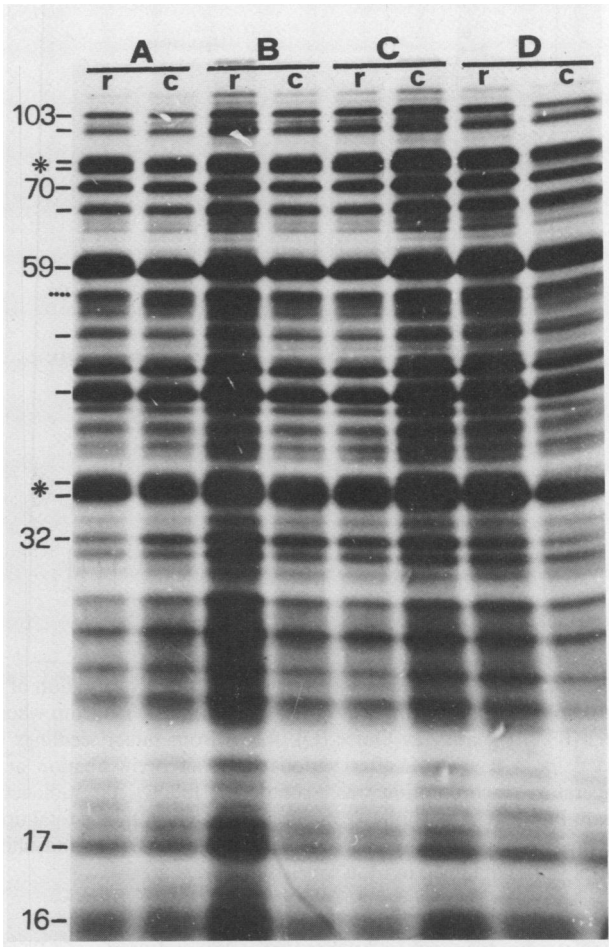


FIG. 8. Protein patterns from heat-shocked roots (r) and coleoptiles (c) of common wheat lines. Seedlings were labeled for 3 h at 40°C after 4 h preincubation at this temperature. Proteins were separated on a SDS gel containing 15% acrylamide, 0.12% bis-acrylamide in the upper half and 20% acrylamide, 0.16% bis-acrylamide in the lower one. Bars and associated number in the left margin indicate major early HSPs and the molecular mass in kD. Asterisks denote HSPs doublets that appeared as single bands in other gels. Lanes: A, common wheat line 7BL; B, line 6BS; C, line 6DS; D, line 7DL.

Cooper and Ho (5) have observed that in maize the induction of HSPs is biphasic, synthesis of 10 early HSPs and then 3 late HSPs. The presence of a third phase of HSPs induction was not observed, possibly because the treatments were stopped after 10 h of heat shock. An interesting aspect of the induction of the first set of late HSPs is their apparent association with an increased rate of protein synthesis. The physiological significance of these late HSPs is unknown and it would be interesting to determine whether they play a thermoprotective role. A second aspect regarding late HSPs is that their appearance in both roots and coleoptiles is variable. It is not yet clear why synthesis of late HSPs cannot be induced in some cultivars or lines. This could be attributed to damage of the protein synthesis machinery by heat as indicated by the very low percentage of radioactive methionine incorporated into proteins after 6 h of incubation at 40°C in cultivars or lines devoid of late HSPs. They could be more sensitive to high temperature than other cultivars; thus, experiments are planned to determine if induction of late HSPs can be obtained using lower temperatures. Cooper and Ho (5) have reported a similar variability in late HSPs synthesis in maize.

Finally, it would be interesting to investigate further the synthesis of early HSP 62. This band is present in all cereal species analyzed and is strongly induced at the beginning of the treatment at high temperature. Synthesis of HSP 62 is transient and occurs only for a short time as indicated by the absence of this protein in seedlings preincubated for 2 h or more prior to labeling with radioactive methionine. However, the appearance of HSP 62 is variable. Two hypotheses can be formulated as to this variability. HSP 62 could be synthesized during a very short interval at the beginning of the heat shock so that it is well labeled during limited periods. HSPs with this kinetics of synthesis are known in mesophyll protoplasts of tobacco (14). Alternatively, HSP 62 could be a protein whose synthesis is a function of temperature and it is possible that the temperature of 40°C used in the heat shock experiments is not optimal for its induction. HSPs whose induction is strictly temperature-dependent have been described (5).

In all inbred lines of maize analyzed by Sinibaldi and Turpen (17) an abundant heat-induced polypeptide of approximately 60 kD was found to be synthesized by the mitochondria. This protein of unknown function appears to correspond to HSP 59,

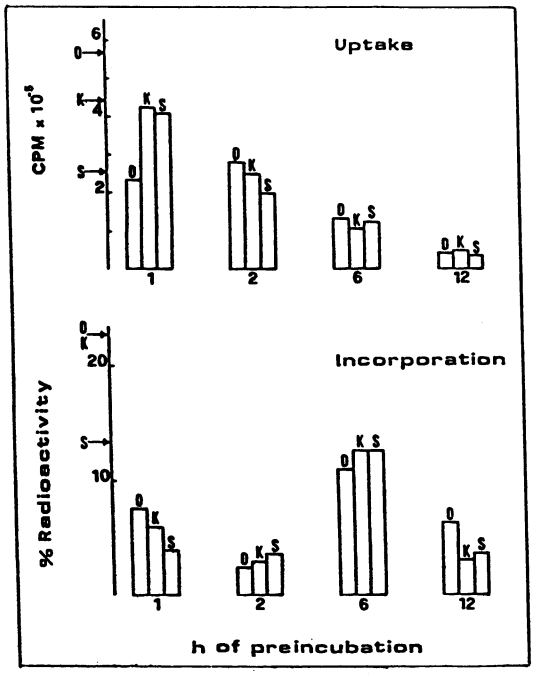


FIG. 9. Effect of heat shock on the uptake and incorporation of [^{35}S] methionine by roots of common wheat cv Salmone (S), durum wheat cv Karel (K), and barley cv Onice (O). Roots from intact seedlings were labeled for 3 h at 40°C after various times of preincubation at this temperature. Incorporation was estimated as follows: the radioactivity present in the TCA-precipitable material was divided by the total uptake of radioactive methionine and multiplied by 100. Arrows indicate the control values obtained in roots labeled for 3 h at 20°C.

which we have found in both coleoptiles and roots of all cereal species analyzed. In 41°C-treated corn seedlings the same authors observed a second prominent band of 62 kD whose resemblance to early HSP 62 is noteworthy. This maize protein, most likely etioplast-encoded (17), was not included among the heat-induced proteins because a band having a similar migration on SDS gels was also found in the 25°C-treated seedlings. However, it is possible that the 62 kD band is a heat-induced polypeptide comigrating with 25°C proteins. If this is the case, it is worth noting that the presence of the 62 kD band in heat-shocked maize seedlings appears to be variable and hardly detectable in some autoradiographs shown by Sinibaldi and Turpen.

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Table I. *Incorporation of Methionine after 6 h of Preincubation at 40°C and Synthesis of Late HSPs in Cereal Species*

Cereal	Cultivar or Lines	Late HSPs	Incorporation of Methionine
			% *radioactivity in protein*
Barley	Onice	+[a]	10.9
Durum wheat	Karel	+	12.2
	Creso	+	6.3
Common wheat	Salmone	+	12.2
	Line 4B4D	+	11.0
	L. 1BL	+	5.9
	L. 3DS	+	6.6
	L. 4BL	+	7.5
	Salmone	–[b]	3.3
	Manital	–	1.6
	Chinese Spring	–	2.6
	L. 1AL	–	2.5
	L. 1DL	–	2.6
	L. 1BS	–	2.0
	L. 5BL	–	1.5

[a] Present. [b] Absent.

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