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Gene induction and repression by salt treatment in roots of the salinity-sensitive Chinese Spring wheat and the salinity-tolerant Chinese Spring × *Elytrigia elongata* amphiploid

(gene expression/mRNA/*in vitro* translation/stress tolerance/interspecific hybrid)

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ABSTRACT **An artificial amphiploid from a cross between salinity-sensitive bread wheat cultivar Chinese Spring and highly tolerant *Elytrigia elongata* (Host) Nevski (= *Agropyron elongatum* Host) shows enhanced salinity tolerance relative to Chinese Spring. Poly(A)$^+$ RNA was isolated from roots, expanding leaves, and old leaves from amphiploid and Chinese Spring plants prior to and after acclimation to high levels of NaCl in solution cultures. Two-dimensional gel electrophoresis of the *in vitro* translation products was used to compare these mRNA populations. The amphiploid had 10 mRNA species induced or enhanced and 8 species repressed in root tissue during acclimation to saline growth conditions. These 18 transcripts affected by salt treatment were also detected in wheat roots, but only 4 of these were similarly regulated. In Chinese Spring the acclimation to saline stress resulted in a marked change in the level of expression of 34 transcripts in root tissue; of these, 26 were detected in the amphiploid and only 6 were regulated as in the amphiploid. No differences were seen in gene expression between salt-treated and control plants in leaves and meristematic crowns and unexpanded leaves of the amphiploid.**

A number of species of the genus *Elytrigia* (= *Agropyron sensu lato*) are known to be highly salt tolerant (1, 2). Some naturally occur in the littoral zones and salt marshes of the Mediterranean and Black Sea regions. Since they are related to cultivated wheats, there has been interest in them as a source of genes for the improvement of salinity tolerance of these crops. An octoploid amphiploid has been derived from a cross between *Elytrigia elongata* Host ($2n = 2x = 14$) and hexaploid *Triticum aestivum* L. cv. Chinese Spring ($2n = 6x = 42$). From this amphiploid extensive stocks of *E. elongata* chromosome additions and substitution to *T. aestivum* have been obtained (3–6). This amphiploid has been shown to be highly tolerant to excessive levels of Na^+, Cl^-, Mg^{2+}, SO_4^{2-}, and sea salt (7), and several of the chromosome addition and substitution lines have been shown to be more salinity tolerant than Chinese Spring (J.D., unpublished). The expression of this tolerance in a *T. aestivum* background is highly fortuitous, as many desirable traits of wheat relatives are not expressed in artificial amphiploids, most likely because of the hexaploidy of *T. aestivum*. The expression of salinity tolerance of *E. elongata* and related *Elytrigia pontica* (Pobp.) Holub in wheat backgrounds (7, 8) indicates that tolerance is genetically dominant over sensitivity and that *Elytrigia* genes could be useful for the improvement of salinity tolerance not only of cultivated wheats but possibly of other grasses, especially in light of the recent report of successful transformation of monocotyledenous species (9).

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The underlying physiological mechanisms of the salinity tolerance of *Elytrigia* are poorly understood, but, in general, even highly salt-tolerant species are observed to have reduced growth rates when cultured under saline stress (for review, see ref. 10). It seems, therefore, reasonable to assume that because this apparent metabolic cost of salt affected growth, the genes controlling tolerance of salinity stress may not be normally active unless the plant is stressed. Our preliminary evidence suggested that that may be the case for the Chinese Spring × *E. elongata* amphiploid (11). The objective of the present study was to obtain more extensive evidence on gene regulation by saline stress in the roots, the meristematic crown, and unexpanded leaves, and in older expanded leaves of the salt-tolerant amphiploid and the salt-sensitive Chinese Spring.

MATERIALS AND METHODS

Seeds of the amphiploid *T. aestivum* cv. Chinese Spring × *E. elongata* and of Chinese Spring wheat were germinated on slant boards in water and transplanted to 150-liter solution culture tanks (≈60 plants per tank). Seedlings were grown for 4 weeks in a greenhouse in modified Hoagland (12) solution containing 3 mM KNO_3, 1 mM $NH_4H_2PO_4$, 0.5 mM $MgSO_4$, 5.5 mM $Ca(NO_3)_2$, 50 mg of FeEDTA per liter (10% iron), 25 μM KCl, 12.5 μM H_3BO_3, 1 μM $MnSO_4$, 1 μM $ZnSO_4$, 0.25 μM $CuSO_4$, and 2 μM H_2MoO_4. After 1 month amphiploid plants were transferred to modified Hoagland solution containing 50 mM NaCl. The NaCl concentration of the growth solution was subsequently increased 50 mM per day to a final concentration of 250 mM, which was maintained for 2 days. Chinese Spring wheat plants, which are less salt tolerant, were transplanted to modified Hoagland solution containing 50 mM NaCl and the NaCl concentration was increased to 75, 100, 125, 150, 175, and 180 mM during the following 6 days. Control amphiploid and Chinese Spring wheat plants were maintained in modified Hoagland solution without NaCl.

Total RNA was extracted separately as described (13) from roots, leaves, and the combined sample of the meristematic crown and unexpanded leaves from inside the basal 2 cm of the tiller, with the modification that the Tris buffer concentration in the lysis buffer was increased to 200 mM. Poly(A)$^+$ RNA was isolated with an oligo(dT)-cellulose column (P-L Biochemicals) as described (14), using a loading buffer of 20 mM Tris, pH 7.6/0.5 M LiCl/1 mM Na_2EDTA/0.1% $NaDodSO_4$; after the sample was loaded on the column, the column was rinsed with 5 vol of loading buffer followed by 5 vol of loading buffer containing 0.1 M LiCl. The eluting buffer was 10 mM Tris, pH 7.6/1 mM EDTA/0.05% $NaDodSO_4$.

A wheatgerm (General Mills, Vallejo, CA) extract for *in vitro* translation was prepared as described (15). Poly(A)$^+$

Abbreviation: IEF, isoelectric focusing.

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RNAs were translated at 28°C for 90 min with [^{35}S]methionine (Amersham; 15 μCi/μg of mRNA; 1 Ci = 37 GBq). Translation mixtures were subsequently digested with ribonuclease A (Sigma) for 15 min at 37°C and precipitated with 90% acetone in aqueous solution; they were then dried and dissolved in a buffer described in ref. 16, which is 9.8 M urea/2% Nonidet P-40/2% Ampholines, pH 3.5–10 (LKB)/1% 2-mercaptoethanol. Translation products were separated by isolectric focusing (IEF)/NaDodSO$_4$/polyacrylamide two-dimensional electrophoresis (17), with modifications (16). IEF gels were 4% polyacrylamide (94.6% acrylamide/5.4% bisacrylamide)/9.8 M urea, with 7.3 μl of pH 3.5–10 Ampholines per ml and 14.6 μl of pH 5–7 Ampholines per ml (LKB); gels were 130 × 1 mm tube gels. Samples were focused at 800 V for 12 hr. IEF gels were extruded into equilibration buffer of 81 mM Tris/2.3% NaDodSO$_4$/5% 2-mercaptoethanol/10% glycerol and either used directly or frozen in a dry ice/methanol bath and stored at −70°C. In either case gels were equilibrated by gentle shaking for 15 min in equilibration buffer before loading onto the second-dimension gels. For electrophoresis in the second dimension, a 170 × 250 × 1 mm slab gel of 11% polyacrylamide (97.3% acrylamide/2.7% bisacrylamide)/0.1% NaDodSO$_4$/0.37 M Tris, pH 8.8 used. The stacking gel was 5% polyacrylamide (with 2.7% bisacrylamide)/0.1% NaDodSO$_4$/0.163 M Tris, pH 6.8. IEF gels were attached to NaDodSO$_4$ slab gels with 1% agarose dissolved in equilibration buffer. NaDodSO$_4$ tank buffer was 0.1% NaDodSO$_4$/0.1 M glycine/0.025 M Tris. NaDodSO$_4$ gels were run at room temperature at 27 mA with maximum voltage of 400 V until the bromphenol tracking dye reached the end of the gel, usually about 7 hr. Gels were stained with Coomassie blue, destained, transferred onto Whatman 3 MM paper, dried in a vacuum dryer, and autoradiographed with Kodak XAR-5 film.

RESULTS

The comparison of autoradiographs of the *in vitro* translation products from the poly(A)$^+$ RNA fractions isolated from the roots of salt-treated and control amphiploid plants showed altered expression of 18 transcripts (Figs. 1 and 2; Table 1). Five transcripts that were not observed in the control plants were induced by the salt treatment; 5 transcripts that were expressed in salt-treated plants and in the controls were expressed at an enhanced level in the salt-treated plants. Eight transcripts were repressed in salt-treated plants. Of these 18 salt-regulated transcripts, 16 were also readily detected in Chinese Spring (Figs. 3 and 4; Table 2). Five (spots 7–10 and 37) were observed to have similar regulation in wheat to that observed in the amphiploid, and 10, though present, were expressed constitutively with respect to salt treatment (Table 2). One (spot 3) that was repressed by salt treatment in the amphiploid was expressed at an enhanced level in Chinese Spring. Two spots (13 and 14) from genes repressed by salt treatment in the amphiploid that were not observed in Chinese Spring wheat were minor spots in the gels; because they were barely detectable, their absence in Chinese Spring cannot be claimed with a high degree of confidence.

In contrast to the gels from the amphiploid, the gels from Chinese Spring showed 34 transcripts that were modified in their expression by salt treatment (Table 2). Sixteen of these transcripts were present in salt-treated plants but were absent in the treated controls and were presumably transcripts of genes induced by salt treatment. Seven additional spots were enhanced, six were absent, and five were greatly reduced in salt-treated plants relative to the control.

Comparison of autoradiograms derived from mRNA from expanded leaves and from unexpanded leaves and the crown meristem showed no differences in the mRNA pools isolated

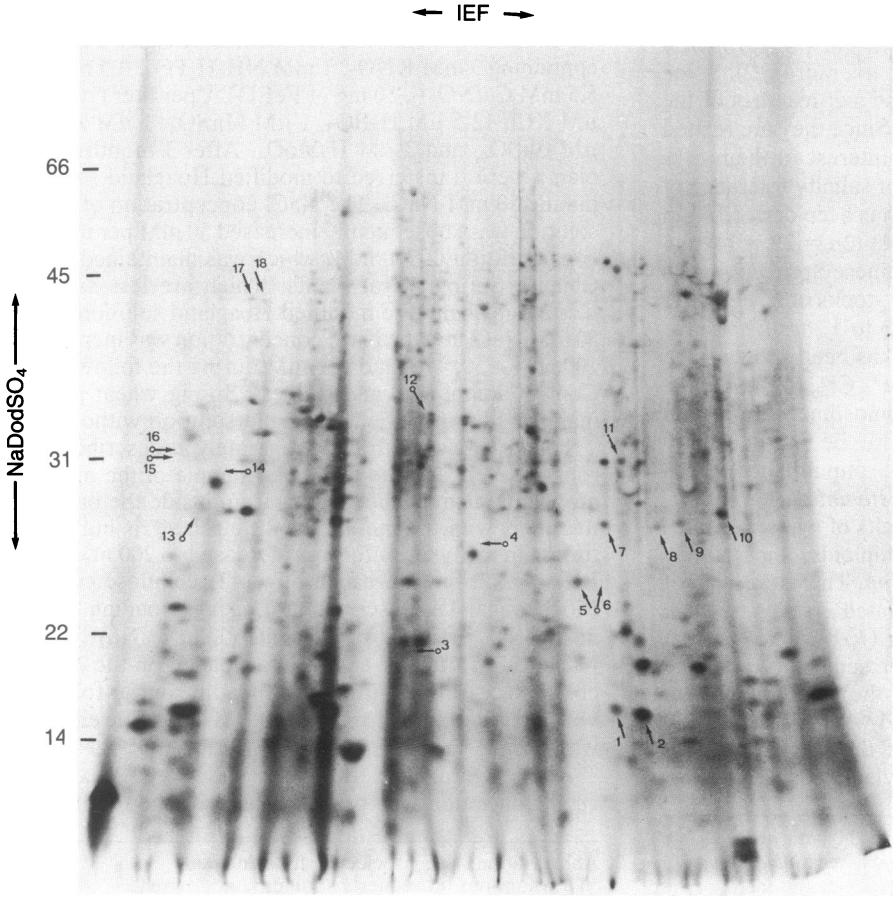

FIG. 1. Autoradiograph of two-dimensional IEF/NaDodSO$_4$/polyacrylamide gel of *in vitro* translation products from the poly(A)$^+$ RNA population isolated from the roots of salt-treated amphiploid Chinese Spring × *E. elongata*. Molecular weights are given as $M_r \times 10^{-3}$. Arrows indicate proteins transcribed from mRNAs induced or whose abundance was increased by salt treatment. Arrows with circles indicate proteins or the location of proteins from mRNAs that were repressed or whose abundance was decreased by salt treatment. The numbers correspond to proteins listed in Table 1.

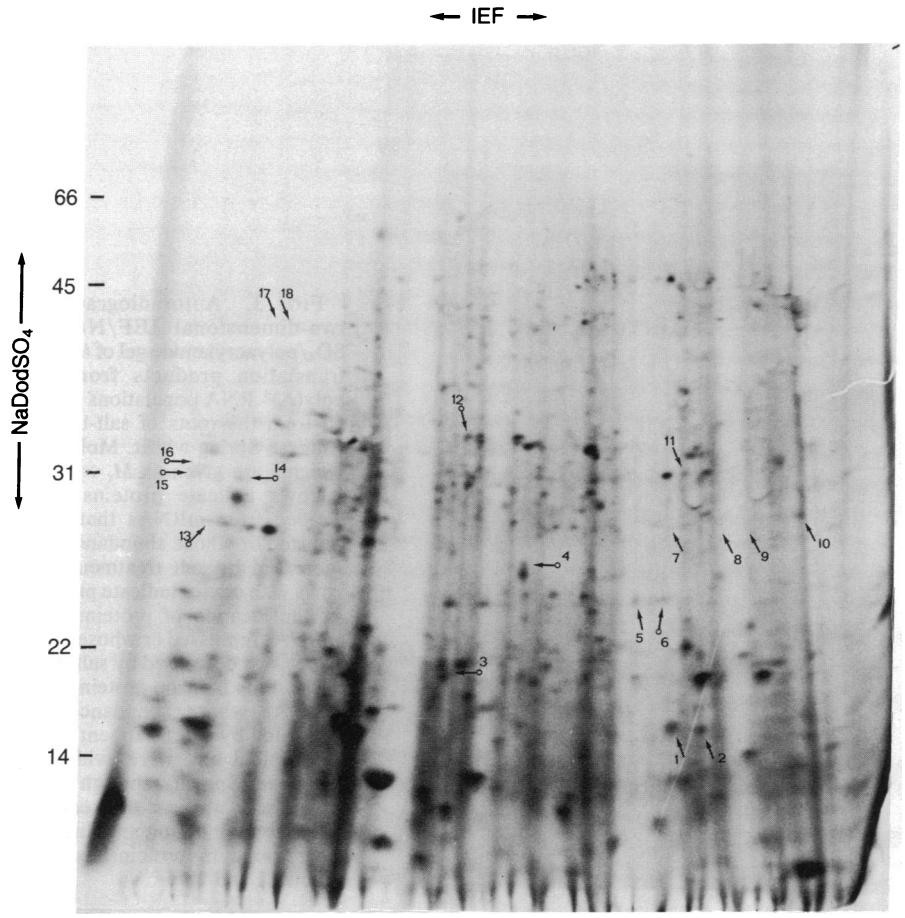

FIG. 2. Autoradiograph of two-dimensional IEF/NaDodSO$_4$/polyacrylamide gel of *in vitro* translation products from the poly(A)$^+$ RNA population isolated from the roots of amphiploid Chinese Spring × *E. elongata* not treated with salt. Molecular weights are given as $M_r \times 10^{-3}$. Arrows indicate proteins or the location of proteins transcripted from mRNAs induced or enhanced by salt treatment. Arrows with circles indicate proteins from mRNAs that were repressed or whose abundance was decreased by salt treatment. The numbers correspond to those in Fig. 1 and to the proteins listed in Table 1.

from salt-treated and control amphiploid and Chinese Spring plants.

DISCUSSION

The acclimation of Chinese Spring wheat and the Chinese Spring × *E. elongata* amphiploid to growth under saline stress elicits a modification of gene expression in these plants that is detected by changes in the relative abundances of several mRNA species. Roots appear to be the major site of change of gene expression in response to saline stress. This is not unexpected since the roots are the organ in direct contact with the saline environment. The failure to detect any changes in the expression in the aboveground organs of both genotypes agrees with the finding of other authors (18) that these plants exclude salt from the shoot, but these findings are nevertheless surprising in view of the numerous changes found in root tissue. It is possible that mRNA species induced in shoots are of low abundance that could escape detection in the autoradiograms.

Table 1. *In vitro* translation products from salt-regulated mRNAs in the Chinese Spring wheat × *E. elongata* amphiploid

Designation in Figs. 1–4	$M_r \times 10^{-3}$	Regulation in amphiploid	Presence in Chinese Spring	Regulation in Chinese Spring
1	16	Increased	Yes	Not regulated
2	16	Increased	Yes	Not regulated
3	19	Repressed	Yes	Increased
4	25	Repressed	Yes	Not regulated
5	24	Increased	Yes	Not regulated
6	24	Repressed	Yes	Not regulated
7	27	Induced	Yes	Induced
8	27	Induced	Yes	Induced
9	27	Induced	Yes	Induced
10	28	Increased	Yes	Increased
11	31	Increased	Yes	Decreased
12	33	Repressed	Yes	Not regulated
13	28	Repressed	?	?
14	30	Repressed	?	?
15	31	Repressed	Yes	Not regulated
16	31	Repressed	Yes	Not regulated
17	42	Induced	Yes	Not regulated
18	42	Induced	Yes	Not regulated

Although the amphiploid carries the genome of *E. elongata*, which is not present in Chinese Spring, none of the salt-affected gene products of the amphiploid appeared unique to the amphiploid; all of the readily observable spots whose expression was affected by salt stress were also detected in Chinese Spring and differed from Chinese Spring only in their regulation. Two minor salt-regulated spots that were observed only in the amphiploid were too faint to claim with confidence that they were not also present in Chinese Spring. However, it cannot be concluded that the salt-regulated spots found in the amphiploid are exclusive products of the *T. aestivum* genomes. There is ample evidence in isozyme and DNA hybridization studies that *Triticum* and *Elytrigia* are closely related molecularly (6, 19, 20). It is possible that *in vitro* translation products from related genes from wheat and *E. elongata* genomes comigrated in the gels. In this context, it would be of interest to determine the relationships of *E. elongata* translation products to those of Chinese Spring wheat and the amphiploid under similar salinity regimes.

The fact that twice as many genes are affected by salt stress in Chinese Spring as in the amphiploid, although Chinese Spring was exposed to slightly lower salinity than the

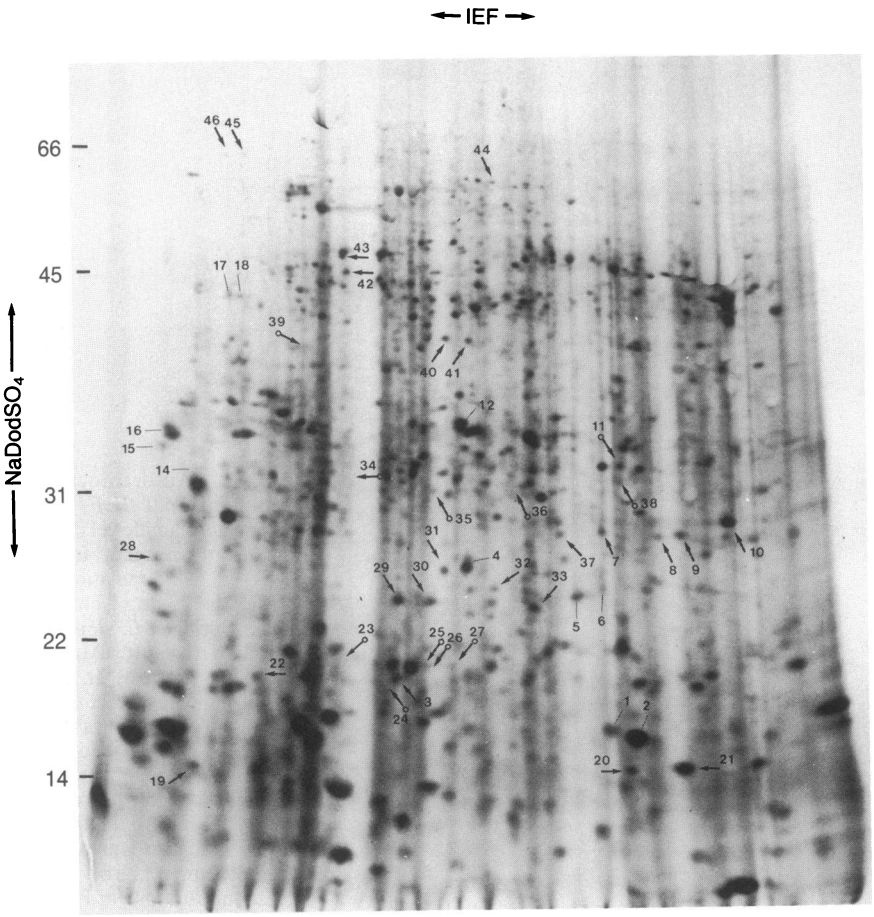

FIG. 3. Autoradiograph of two-dimensional IEF/NaDodSO$_4$/polyacrylamide gel of *in vitro* translation products from the poly(A)$^+$ RNA populations isolated from the roots of salt-treated Chinese Spring wheat. Molecular weights are given as $M_r \times 10^{-3}$. Arrows indicate proteins transcribed from mRNAs that were induced or whose abundance was increased by salt treatment. Arrows with circles indicate proteins or the location of proteins from mRNAs repressed or whose abundance was decreased by salt treatment. Bars indicate proteins from mRNAs whose abundance was regulated by salt treatment in the amphiploid and were evident in Chinese Spring, though their expression was not markedly affected by salt treatment. Numbers correspond to proteins listed in Tables 1 and 2.

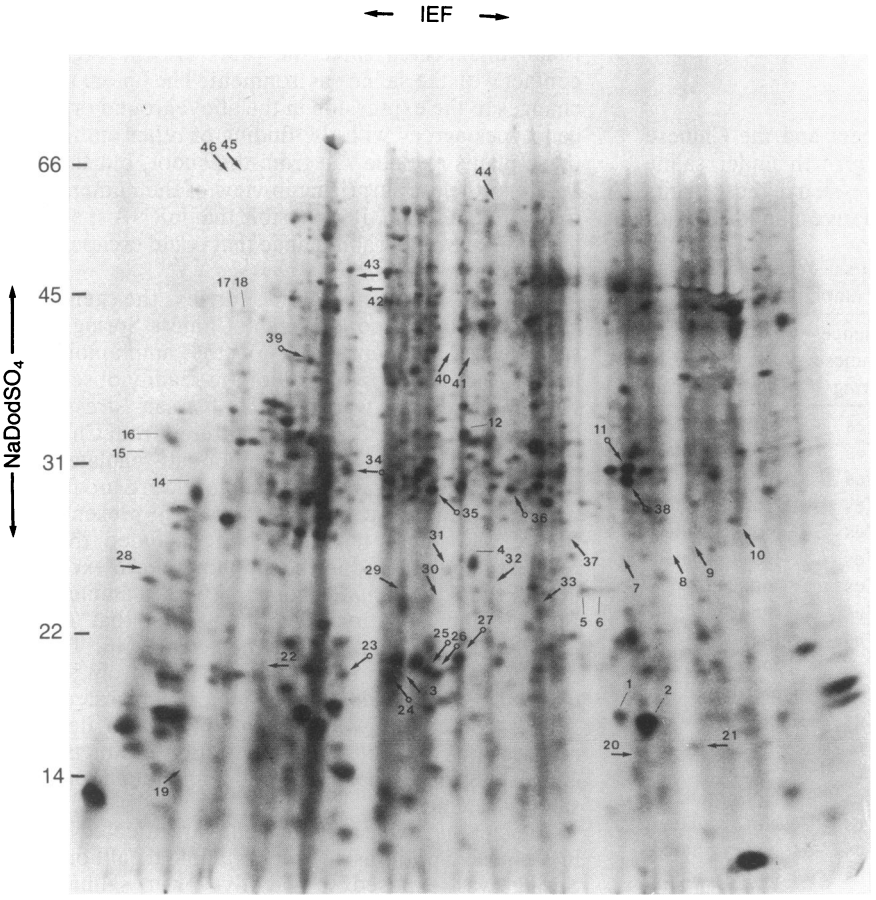

FIG. 4. Control Chinese Spring: autoradiograph of two-dimensional IEF/NaDodSO$_4$/polyacrylamide gel of *in vitro* translation products from the poly(A)$^+$ RNA population isolated from the roots of Chinese Spring wheat not treated with salt. Molecular weights are given as $M_r \times 10^{-3}$. Arrows indicate proteins or location of proteins from mRNAs that were induced or whose intensity was increased by salt treatment. Arrows with circles indicate proteins from mRNAs that were repressed or whose abundance was decreased by salt treatment. Bars indicate proteins from mRNAs whose abundance was regulated by salt treatment in the amphiploid and were evident in Chinese Spring, though their expression was not markedly affected by salt treatment. The numbers correspond to those of Fig. 3 and to the proteins listed in Tables 1 and 2.

Table 2. *In vitro* translation products of salt-regulated mRNAs from Chinese Spring wheat

Designation in Figs. 1–4	$M_r \times 10^{-3}$	Regulation in wheat	Presence in amphiploid	Regulation in amphiploid
19	15	Induced	No	—
20	15	Increased	Yes	Not regulated
21	15	Increased	Yes	Not regulated
22	19	Increased	Yes	Not regulated
23	19	Decreased	Yes	Not regulated
24	19	Repressed	No	—
3	19	Increased	Yes	Repressed
25	19	Repressed	No	—
26	17	Repressed	Yes	Not regulated
27	20	Repressed	Yes	Not regulated
28	25	Induced	Yes	Not regulated
29	22	Increased	Yes	Not regulated
30	22	Induced	Yes	Not regulated
31	24	Induced	No	—
32	23	Increased	Yes	Not regulated
33	22	Induced	Yes	Increased slightly
34	31	Repressed	Yes	Not regulated
35	29	Decreased	No	—
36	30	Repressed	No	—
37	27	Induced	Yes	Induced
7	28	Induced	Yes	Induced
38	31	Decreased	Yes	Increased slightly
11	31	Decreased	Yes	Increased
8	27	Induced	Yes	Induced
9	27	Induced	Yes	Induced
10	28	Increased	Yes	Increased
39	39	Decreased	Yes	?
40	40	Induced	Yes	Not regulated
41	40	Induced	Yes	Not regulated
42	45	Induced	Yes	Not regulated
43	47	Induced	No	—
44	60	Induced	?	?
45	65	Induced	Yes	?
46	65	Induced	Yes	?

amphiploid, suggests that a number of genes in Chinese Spring are affected by the altered metabolism due to salt stress that have little or nothing to do with the control of salt tolerance. Because the amphiploid is more salt tolerant than Chinese Spring, the most important question is whether any of the genes shown to be regulated by salt stress in the amphiploid are causally related to its superior salt stress tolerance. Although the results of experiments reported here cannot address that question, they do establish that a certain limited number of genes are induced by salt stress and that these genes are differentially expressed in the two genotypes. This offers a strategy for selecting these genes from a cDNA library by differential colony hybridization as has been followed in many systems (e.g., heat shock proteins in plants, see ref. 21). Such an isolation of these salt-regulated genes would make it feasible to study the role that these genes might play in saline stress tolerance of wheat, *E. elongata*, and their amphiploid.

Characterization of *in vivo* protein profiles in cultured tobacco cell lines that were selected for adaptation to growth in cultures with high levels of NaCl in the culture media (22) showed altered abundances of several proteins. Whether any of these proteins are homologous to the salt-regulated *in vitro* translation products reported here cannot be determined, especially since *in vitro* translation products are not subject to the same posttranslational processing as *in vivo* proteins. Similarly, *in vitro* translation products are a better reflection of immediate gene activity than *in vivo* protein profiles since different protein species will likely have differing rates of degradation. This consideration is relevant not only to the comparison of the wheat system to the tobacco cell culture system but also to *in vivo* protein profiles of salt-treated Chinese Spring and the amphiploid. In any case, it is doubtful that the comparison of protein profiles of tobacco and wheat could be conclusive; rather, the cloning of induced genes appears to be the best strategy to determine the molecular homology of the proteins induced in these two systems.

Note Added in Proof. Ramagopal has reported on the molecular biology of salinity stress in barley (23).

We thank B. C. Jenkins for his cooperation in providing us with seed of the amphiploid *T. aestivum* cv. Chinese Spring × *E. elongata*.

1. Dewey, D. R. (1960) *Agron. J.* **52,** 631–635.
2. McGuire, P. E. & Dvořák, J. (1981) *Crop Sci.* **21,** 702–705.
3. Dvořák, J. & Knott, D. R. (1974) *Can. J. Genet. Cytol.* **16,** 399–417.
4. Dvořák, J. (1980) *Can. J. Genet. Cytol.* **22,** 237–259.
5. Dvořák, J. & Chen, K. C. (1984) *Can. J. Genet. Cytol.* **26,** 128–132.
6. Hart, G. E. & Tuleen, N. A. (1983) *Genet. Res.* **41,** 181–202.
7. Dvořák, J. & Ross, K. (1986) *Corp Sci.* **26,** 658–660.
8. Dvořák, J., Ross, K. & Mendlinger, S. (1985) *Crop Sci.* **25,** 306–309.
9. Fromm, M. E., Taylor, L. P. & Walbot, V. (1986) *Nature (London)* **319,** 791–793.
10. Greenway, H. & Munns, R. (1980) *Annu. Rev. Plant Physiol.* **31,** 149–190.
11. Gulick, P. J. & Dvořák, J. (1985) *J. Cell. Biochem.*, Suppl. 9C, 217 (abstr.).
12. Hoagland, D. R. & Arnon, D. I. (1950) *Calif. Agric. Exp. Stn. Circ.* **347,** 1–32.
13. Cathala, G., Savouret, J., Mendez, B., Wet, B. L., Karin, M., Marshall, J. A. & Baxter, J. D. (1983) *DNA* **2,** 329–335.
14. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
15. Anderson, C. W., Straus, J. W. & Dudock, B. S. (1983) *Methods Enzymol.* **101,** 635–644.
16. Duncan, R. & Hershey, J. W. B. (1984) *Anal. Biochem.* **138,** 114–155.
17. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
18. Storey, R., Graham, R. D. & Shepherd, K. W. (1985) *Plant Soil* **83,** 327–330.
19. Dvořák, J. & Scheltgen, E. (1973) *Proc. Int. Wheat Genet. Symp.* **4,** 73–79.
20. Appels, R. & Moran, L. B. (1984) *Stadler Genet. Symp.* **16,** 529–557.
21. Schoffl, F. & Key, J. L. (1982) *J. Mol. Appl. Genet.* **1,** 301–314.
22. Sing, N. K., Handa, A. K., Hasegawa, P. M. & Bressan, R. A. (1985) *Plant Physiol.* **79,** 126–137.
23. Ramagopal, S. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 94–98.