Osmotic and Salt Stresses Induced Differential Alteration in Water-Soluble Carbohydrate Content in Wheat Seedlings

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Water-soluble carbohydrates contributing to genotypic differences in response to consecutive drought and salinity stresses in wheat seedlings were investigated. Total water-soluble carbohydrate, glucose, fructose, sucrose, and fructan contents and the distribution of degree of polymerization fructans were measured in wheat seedlings exposed to 18% poly(ethylene glycol) (PEG)-induced drought stress followed by an equiosmolar salinity. Tolerant genotypes accumulated higher soluble carbohydrate levels than the sensitive ones. Both ionic and nonionic stresses increased the percentage distribution of reducing sugars. The concentration of the principal component of soluble carbohydrate content increased in response to drought stress and, conversely, mostly decreased due to salt stress. PEG-induced fructan accumulation was highest in leaves and showed a positive correlation with the drought tolerance of the varieties. Fructan content in stems increased in tolerant genotypes but decreased in sensitive ones under NaCl treatment. Increment of polyfructan percentage distribution was greater in tolerant varieties than in sensitive ones.

Keywords: *Carbohydrates; drought; fructan; salinity; osmotic stress; wheat (Triticum aestivum L.)*

INTRODUCTION

The most prevalent environmental stresses on plants affect water status. Plant species vary in their sensitivity and response to the decrease in water potential caused by drought, low temperature, or high salinity. Biochemical studies have revealed similarities in processes induced by stress that lead to the accumulation of metabolites (McKersie and Leshen, 1994). One way all organisms tolerate abiotic stress to some degree is by accumulation of solutes termed compatible (Munns and Weir, 1981; Hanson and Hitz, 1982; Morgan, 1992; Galiba, 1994; Colmer et al., 1995; Rosa-Ibarra and Maiti, 1995). These metabolites include amino acids, polyamines, and water-soluble carbohydrates. If the primary function of solute accumulation is the regulation of intercellular water activity (osmoregulation), it has been suggested that the role of these solutes is water structure regulation (McKersie and Leshen, 1994). Sugars protect membranes and proteins against dehydration by inducing glass formation at physiological temperature, and polyhydroxyl components may replace the structural water (Santarius and Bauer, 1983; Guy, 1990).

Fructans are linear or branched fructose polymers with glucose, usually as an end unit (inulin, levan). They are highly water-soluble and nonreducing oligo- and polysaccharides synthesized from sucrose by repeated fructosyl transfer. In addition to being a reserve carbohydrate, fructan appears to be advantageous for plants under low-temperature (Bancal and Triboi, 1993; Galiba et al., 1997), drought (Virgona and Barlow, 1991; Hendry and Wallace, 1993), or anoxia (Albrecht et al., 1993) conditions, but there is no evidence of its involvement in protective mechanisms. Fructan content is affected by the source/sink manipulation of the plant, whereas sucrose content is relatively stable, which suggests a buffer role for fructan (Housley and Pollock, 1993). The fact that fructan metabolism involves the principal sugar components (sucrose, fructose, and glucose) may be a possible explanation for its central role in adaptation processes. The enzymes generally considered to be involved in plant fructan synthesis (Edelman and Jefford, 1964) include sucrose-sucrose fructosyltransferase (SST, EC 2.4.1.99), which catalyzes fructosyl transfer from one sucrose molecule to another, leading to 1-kestose and glucose. Regulation of fructan metabolism may be largely controlled by SST and the supply of sucrose. The different fructan-fructan fructosyltransferases (FFT, EC 2.4.1.100) are responsible for chain elongation, catalyzing the transfer of fructosyl residues from one fructan molecule to another. Fructan-exohydrolase (FEH, EC EC.3.2.1.*xx*) was found to be responsible for fructan depolymerization. Fructan can

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Table 1. Properties of Wheat Varieties Used in This Study

variety	tolerance		origin
	drought	salinity	
Sakha-8 (Sa)	tolerant	tolerant	Egypt
Kobomugi (Ko)	tolerant	sensitive	Japan
Chinese Spring (Ch)	moderate	moderate	Hungary
Regina (Re)	sensitive	sensitive	Germany

be stored at different sites within the plant, and very different patterns of synthesis and degradation occur under the influence of a range of external and internal factors. The main distinction can be made between heterotrophic organs, such as roots, stems, and grains, and autotrophic organs such as leaves. Fructan storage in leaves is intimately connected with the synthesis of sucrose; in a heterotrophic sink synthesis is from imported carbon and thus less affected by short-term environmental fluctuation (Housley and Pollock, 1993; Pollock and Cairns, 1991).

The early effect of both drought and salt stresses is to decrease the soil water potential, but in the second phase of salinity the concentrations of toxic ions increase in the cells when the vacuoles can no longer sequester incoming salt. In this second phase genotypes that vary in salt tolerance may respond differently as a result of their different abilities to exclude toxic ions or sequester them in the vacuoles (Munns, 1993).

Although the increase in sugar and fructan content in response to water stress is well-known (Virgona and Barlow, 1991; Hendry and Wallace, 1993; Kameli and Lösel, 1993; Al Hakimi et al., 1995), it is not yet clear why different abiotic conditions favor the accumulation of different osmolytes. A study of biochemical pathways leading to these individual compounds may provide insight into how to engineer plants to tolerate complex environments. Furthermore, studies based on changes in amino acid (Morgan 1992), polyamine (Galiba et al., 1989; Erdei et al., 1990), or sugar content (Munns and Weir, 1981; Kameli and Lösel, 1993; Al Hakimi et al., 1995; Bolarin et al., 1995) suggested a genotype-dependent response to osmotic stresses. These considerations raise the question of whether resistance or sensitivity to salt and drought stresses can be recognized in changes in the concentration of specific sugar components.

Recent works have shown that wheat varieties differing in drought and salt tolerance responded in a genotype-dependent manner with respect to their polyamine biosynthesis in wheat calli (Trivedi et al., 1991; Galiba et al., 1993). Soluble sugar content proved to be a better marker than proline content for selecting improved durum wheat regarding drought tolerance (Al Hakimi et al., 1995). In these experiments only the total sugar content was determined, without the identification of specific sugar components. Changes in net photosynthesis, stomatal conductance, and abscisic acid content were also correlated with the degree of drought and salt tolerance in wheat varieties exposed to consecutive drought and salt stress (Nagy and Galiba, 1995). Furthermore, on the basis of the changes in sucrose and fructan content under low temperature, the chromosomes involved in osmoregulation were identified (Galiba et al., 1997).

In the present paper changes in water-soluble carbohydrate, glucose, fructose, sucrose, and fructan contents under consecutive drought and salt stress conditions are compared in leaves, stems, and roots of wheat seedlings differing in drought and salt tolerance, with the objective of identifying differences in the early traits of drought and salt tolerance.

MATERIALS AND METHODS

Plant Materials. Seedlings of bread-wheat (*Triticum aestivum* L.) varieties were grown in half-strength modified Hoagland's nutrient solution (Nagy and Galiba, 1995) in a plant growth chamber (Conviron, Ontario, Canada) for 2 weeks prior to the stress period. The nutrient solution contained the following chemicals: KNO_3, 5 mM; $Ca(NO_3)_2 \cdot 4H_2O$, 5 mM; $MgSO_4 \cdot 7H_2O$, 2 mM; KH_2PO_4, 1 mM; and NaFe-EDTA, 0.1 mM (Sigma, catalog no. E6760). Micronutrient concentrations were as follows: H_3BO_3, 11.5 μM; $MnCl_2 \cdot 4H_2O$, 4.6 μM; $ZnSO_4 \cdot 7H_2O$, 0.2 μM; $Na_2MO_4 \cdot 2H_2O$, 0.12 μM; and $CuSO_4 \cdot 5H_2O$, 0.08 μM.

Table 1 shows the varieties chosen because of their known responses to drought and salinity (Erdei et al., 1990; Galiba et al., 1989, 1993; Trivedi et al., 1991; Nagy and Galiba, 1995). Day/night (16/8 h) cycles of temperature and dew point (relative humidity) of the ambient air were 15/10 °C and 60/75% with 300 μmol m^{-2} s^{-1} light intensity during the daytime.

Osmotic stress was imposed at the beginning of the third week by the application of PEG 4000 (Sigma) at a concentration of 18% in the nutrient solution, which caused a drop of water potential to 0.5 MPa (Nagy and Galiba, 1995). After 7-day PEG treatment, some plants were transferred either to nutrient solution containing equiosmolar NaCl (200 mM) for 4 days or to Hoagland's solution without any supplements to study the recovery. Control seedlings were grown in Hoagland solution throughout the experiment. Samples of leaf blades, stems (crown plus whorl), and roots were taken 2, 7, and 11 days after treatment.

Chemical Analysis. Total water-soluble carbohydrate (WSC) content was determined on lyophilized plant material (Kerepesi et al., 1996). Samples of 200 mg of dry weight were extracted twice using 40 mL of 80% boiling ethanol for 15 min and twice using 40 mL of boiling water for 15 min. Boiling was performed under reflux, and the fractions were collected and cleared by filtering through Whatman No. 42 paper. The filtrates were dried at 40 °C under reduced pressure and were dissolved in distilled water. Oligosaccharides were hydrolyzed by boiling in 0.5% HCl for 30 min. The amounts of free (analyzed before hydrolysis) and bound (analyzed after hydrolysis) glucose, fructose, and sucrose were measured using enzymatic methods (Boehringer Mannheim Kit 716 260): Glucose was determined by first converting to glucose-6-phosphate in the presence of ATP and hexokinase and then measuring its reduction of NADP in the presence of glucose-6-phosphate dehydrogenase. Absorbance by $NADPH+H^+$ was read at 365 nm. Fructose was phosphorylated as for glucose, converted to glucose-6-phosphate with phosphoglucoisomerase, and then assayed as for glucose. Sucrose was hydrolyzed with invertase, and glucose released by the hydrolyses was assayed as above. WSC were determined with the phenol−sulfuric acid method.

Oligofructans were fractionated on silica gel TLC plates (silica gel, HPTLC 60 F_{254}, layer thickness 0.2 mm, Merck, Darmstadt, Germany) with *n*-butanol/ethanol/water (5:3:2) three times, 20−22 °C (Suzuki, 1989).

Fructans were detected with thymol reagent using inulo-oligosaccharides from tubers of *Helianthus tuberosus* as standard. The lanes were analyzed with a densitometer (Sharp JX-325 Image Master System, Pharmacia Biotech) using fructose calibration curve.

Statistical Analysis. Three replications of each sampling were performed. The data were analyzed with the Statgraphics statistical package, using the *t* test and ANOVA functions to assess significant differences between the means.

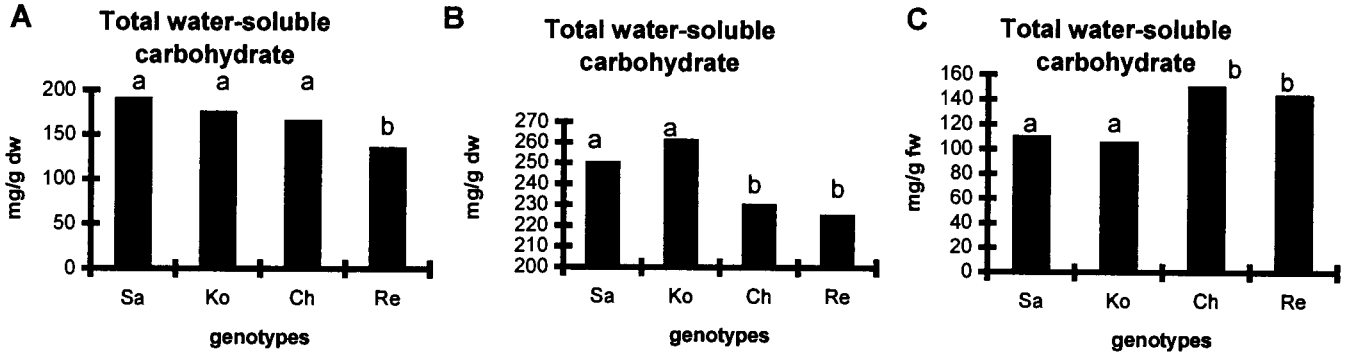

Figure 1. WSC content in the stems (A), leaves (B), and roots (C) of 2-week old seedlings of different wheat genotypes growing in Hoagland's solution. Means within a treatment with different superscript letters were different at the 0.05 probability level.

Table 2. Changes in Water-Soluble Carbohydrate Content (Milligrams per Gram of Dry Weight) in Four Wheat Varieties during Consecutive 18% PEG-Induced Drought Stress and 200 mM NaCl-Induced Salinity

	time (day)	Sakha (mean ± SD)	Kobomugi (mean ± SD)	Chinese Spring (mean ± SD)	Regina (mean ± SD)
stems					
control	2	188.4 ± 20.3	172.2 ± 12.9	164.7 ± 18.3	124.8 ± 11.3
	7	175.7 ± 32.8	168.1 ± 18.9	155.6 ± 20.3	112.9 ± 18.4
PEG	2	369.7 ± 24.6	268.4 ± 22.6	270.8 ± 22.5	220.8 ± 25.2
	7	337.1 ± 28.4	612.1 ± 55.2	383.1 ± 36.7	284.6 ± 25.1
NaCl	11	491.8 ± 32.8	247.5 ± 21.7	372.6 ± 36.1	291.4 ± 30.1
leaves					
control	2	272.56 ± 29.2	288.43 ± 32.1	254.81 ± 31.4	255.12 ± 29.5
	7	251.12 ± 32.1	262.45 ± 31.5	230.89 ± 28.4	225.75 ± 26.2
PEG	2	384.41 ± 43.3	310.55 ± 41.1	450.45 ± 52.6	164.33 ± 21.1
	7	349.23 ± 41.4	513.86 ± 60.2	207.66 ± 25.4	295.54 ± 32.5
NaCl	11	202.46 ± 28.4	209.22 ± 28.5	228.33 ± 41.2	157.79 ± 23.1
roots					
control	2	114.24 ± 15.3	112.44 ± 16.3	158.11 ± 20.3	151.75 ± 20.1
	7	123.56 ± 17.2	103.36 ± 13.5	163.55 ± 19.3	145.23 ± 17.25
PEG	2	177.34 ± 21.3	197.32 ± 22.4	157.85 ± 19.4	88.42 ± 10.56
	7	150.78 ± 18.9	292.44 ± 31.4	172.23 ± 19.4	171.77 ± 19.44
NaCl	11	195.46 ± 23.7	200.34 ± 25.5	262.13 ± 30.1	174.34 ± 25.82

RESULTS

A comparative study on changes in WSC and main sugar components in wheat genotypes differing in drought and salt tolerance was carried out under consecutive PEG and NaCl treatment.

WSC. Differences detected among genotypes in WSC in 14-day-old control seedlings (Figure 1) were related to their stress sensitivity. Stems (Figure 1A) and leaves (Figure 1B) of tolerant cultivars (Sa, Ko, and Ch) contained significantly ($P < 0.05$) more sugar than those of the sensitive cultivar (Re), whereas in roots (Figure 1C) the tendency was inverse.

In all four varieties WSC increased after exposure to PEG-induced water stress (Table 2). The drought tolerant varieties accumulated higher sugar content than the sensitive ones in all plant organs examined. The tolerant Sakha reached its maximum WSC level by day 2, whereas in other varieties continuous sugar accumulation was observed during the PEG treatment. The highest rate of sugar accumulation was detected in the stems (1.5−3-fold). There were similar sugar accumulation rates in roots (1.2−2.2-fold) and in leaves (0.7−2-fold). The sensitive Regina behaved differently from the tolerant ones. It showed an initial decrement in WSC and surpassed control concentrations only after day 7 of the PEG treatment.

On transferring of plants from PEG to NaCl, the WSC content increased in the stems and roots of salt-tolerant varieties (1.3−1.5-fold) and decreased (0.4−0.7-fold) or showed little changes in sensitive or somewhat tolerant ones. The WSC concentration decreased (0.4−0.6-fold) in the leaves of all varieties under NaCl stress.

Sugar Components. Changes in sugar content in response to PEG and NaCl stresses were different in tolerant and sensitive varieties. These tendencies are represented by the results obtained for Sakha and Regina varieties in Figure 2. Considering the components of the soluble carbohydrates, our results suggest that the initial reaction to PEG in the stems and leaves of the tolerant genotypes was an increase in the percentage of monosaccharides (from 45 to 63%) and a decrease in fructan (from 30 to 20%), whereas in the root there was an increase in the rate of sucrose (from 18 to 25%). At the seventh day of PEG treatment fructan showed the highest accumulation rate (50−60%) in shoots (leaf plus stem), and glucose, fructose, and sucrose showed the highest accumulation rate (70−80%) in roots. The main differences in the distribution of sugar components between tolerant and sensitive varieties were detected in stems: in the case of Regina the initial response was a slight decrease in glucose, fructose, and sucrose (from 50 to 30%) and an increase in fructan (from 35 to 50%) followed by an increase in the rate of monosaccharides and a decrease in fructan at day 7.

NaCl treatment caused considerable changes both in the amount and in the rate of sugar components. In tolerant and moderately tolerant genotypes the rate of fructan increased both in stems (from 50 to 60%) and in roots (from 10 to 50%), whereas glucose and fructose decreased (root, from 60 to 25%; stem, from 21 to 15%). On the other hand, in sensitive genotypes the most typical change was an increase in fructose and a decrease in fructan both in stems (from 21 to 38%, from

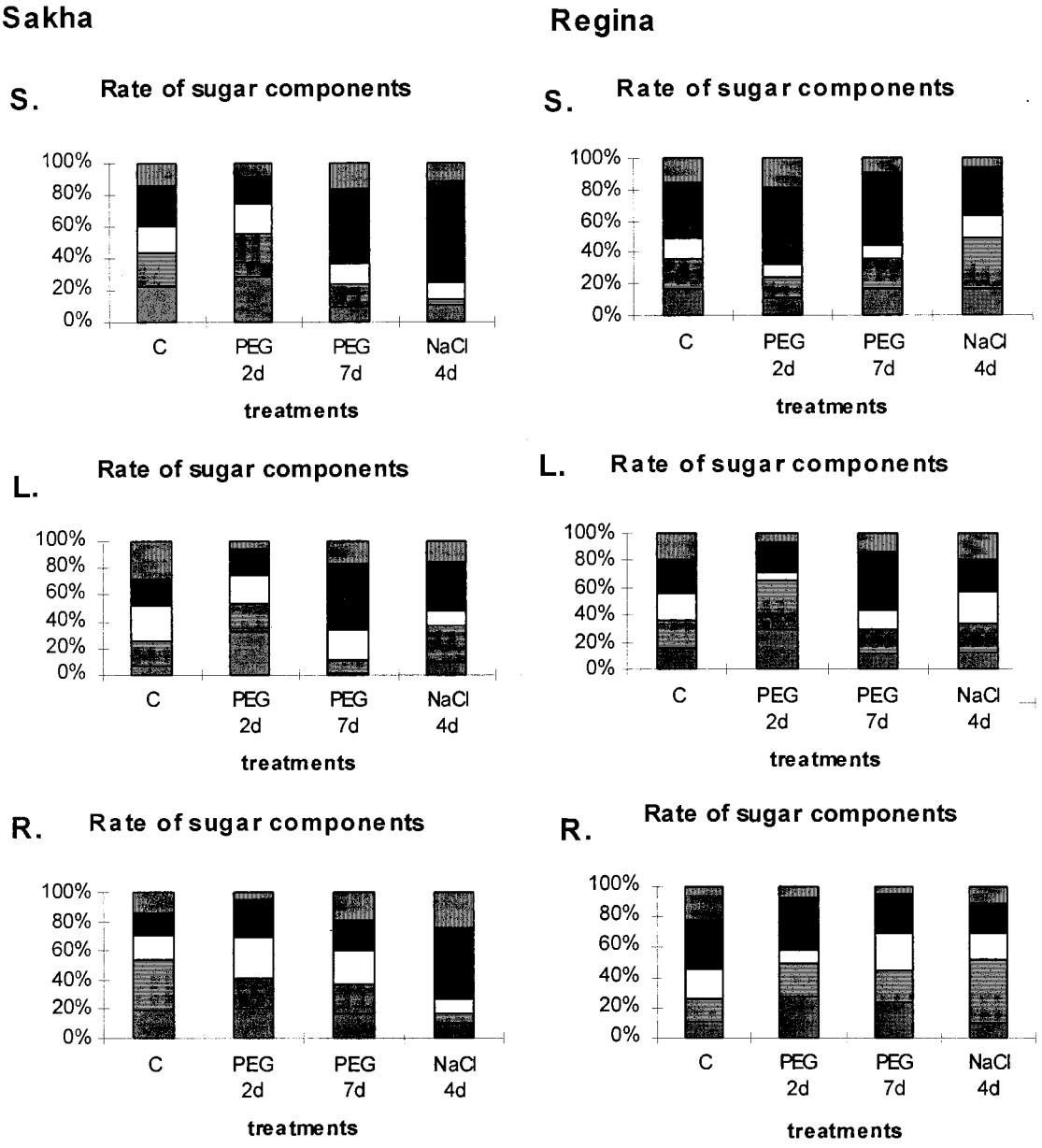

Figure 2. Changes in glucose, fructose, sucrose, and fructan levels in the percentage of WSC in wheat seedlings of the varieties Sakha and Regina, grown in 18% PEG-treated culture solution until day 7, followed by a transfer to equiosmolar (200 mM) NaCl solution until day 11: S, stem; L, leaf; R, root. The bars are, from bottom to top, glucose, fructose, sucrose, fructan, and glucan.

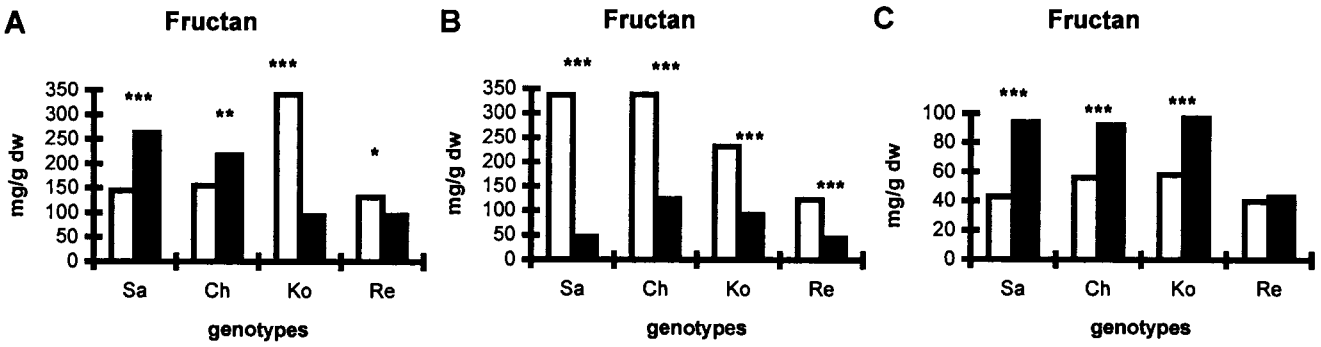

Figure 3. Fructan concentration in the stems (A), leaves (B), and roots (C) of four wheat varieties grown in 18% PEG containing Hoagland's solution until day 7 and then transferred to equiosmolar (200 mM) NaCl solution until day 11: □, PEG; ■, NaCl. Differences from control were significant at the $P < 0.05$ (*), < 0.01 (**), and < 0.001 (***) levels.

47 to 32%) and in roots (from 21 to 42%, from 29 to 20%). In leaves the most characteristic changes were a decreasing rate in fructan (Sa, from 70 to 35%; Re, from 47 to 22%) accompanied by an increase in fructose (from 5 to 20%) in the tolerant varieties and by an increase in sucrose (from 14 to 26%) in the sensitive ones.

Table 3. Changes in Fructan Content (Milligrams per Gram of Dry Weight) in the Stems of Four Wheat Varieties on Days 2 and 7 of 18% PEG Treatment

	time (day)	Sakha (mean ± SD)	Kobomugi (mean ± SD)	Chinese Spring (mean ± SD)	Regina (mean ± SD)
stems					
control	2	68.6 ± 7.3	55.1 ± 6.2	54.2 ± 7.1	49.7 ± 6.2
	7	60.1 ± 5.7	51.8 ± 6.2	50.9 ± 7.8	47.6 ± 5.3
PEG	2	135.2 ± 21.7	45.2 ± 7.1	75.2 ± 6.3	107.3 ± 12.8
	7	144.8 ± 25.6	340.1 ± 40.6	154.5 ± 20.8	131.3 ± 15.5
leaves					
control	2	80.12 ± 9.34	42.76 ± 5.37	53.65 ± 6.31	57.12 ± 6.44
	7	85.59 ± 8.76	45.22 ± 5.22	57.11 ± 6.54	52.18 ± 8.18
PEG	2	170.55 ± 19.3	58.24 ± 6.13	211.3 ± 25.7	33.79 ± 4.25
	7	337.12 ± 40.3	232.61 ± 31.4	338.4 ± 38.11	123.67 ± 15.4
roots					
control	2	61.33 ± 3.78	39.44 ± 3.11	43.12 ± 5.55	46.11 ± 4.12
	7	65.55 ± 3.11	36.52 ± 3.55	47.83 ± 5.56	46.82 ± 3.55
PEG	2	31.69 ± 3.55	29.77 ± 6.89	32.42 ± 5.44	33.19 ± 3.89
	7	42.58 ± 3.02	57.79 ± 7.45	55.79 ± 5.78	39.76 ± 5.48

Fructan. Because of its central role in the response to osmotic stress, the changes in fructan content during PEG and NaCl treatments are presented here in detail.

The initial fructan levels were similar in all varieties examined (Table 3) except Sakha, which contained the higher amount. There were no detectable differences in the fructan content of stems, leaves, and roots of single control plants.

The fructan concentration increased continuously in all varieties both in the stems (2.4−6.6-fold) and in the leaves (2.3−5.9-fold) during the PEG treatment. The highest fructan level was measured in the leaves and was proportional to the degree of drought tolerance of the varieties (Table 3; Figure 3B). In roots PEG treatment decreased the fructan concentration at day 2, followed by an increasing trend at day 7.

Changes in fructan content of the seedlings following the transfer from PEG to a NaCl medium were also different in leaves, stems, and roots (Figure 3). In stems, changes in fructan content was positively correlated with the degree of salt tolerance (Figure 3A): it increased in salt-resistant (Sa) and moderately tolerant (Ch) varieties, whereas it decreased in sensitive ones (Ko, Re). In leaves of all four varieties a sharply decreasing fructan level was detected (Figure 3B). At the same time the fructan accumulation in roots increased significantly except in the case of Re, which showed similar fructan concentration under both ionic and nonionic stresses.

TLC analysis followed by densitometrical evaluation was used to determine the oligofructan concentration (DP < 10) and to calculate its percentage of the total fructan content. Our results clearly show that in the control plants fructans of DP < 10 represented the total fructan content (Table 4). The effect of both ionic and nonionic osmotic stresses increased the rate of polyfructans. In this respect leaves proved to be the most sensitive to PEG treatment, whereas roots were most sensitive to salinity. The initial increase in the polyfructan concentration of leaves was related to the degree of drought tolerance of the genotypes. On transferring the plants back to control solution, the original fructan patterns were regenerated at day 4 in the tolerant varieties. Among the plant organs, the slowest recovery was observed in roots.

Table 4. Changes in the Percentage of Oligofructan in the Total Fructan Content of Wheat Varieties Exposed to Consecutive PEG (18%) Treatment and Salinity (200 mM)

genotype		control	PEG day 2	PEG day 7	PEG → NaCl day 11	recovery day 4[a]
Sakha	stems	91.1	96.0	81.1	88.4	89.0
	leaves	98.3	35.3	26.9	100.0	100
	roots	97.5	89.3	96.3	30.4	91.2
Kobomugi	stems	87.8	91.5	58.7	41.8	97.7
	leaves	85.6	53.1	80.9	23.5	97.2
	roots	94.2	85.1	86.1	8.5	54.8
Chinese Spring	stems	89.3	91.8	30.0	26.7	83.1
	leaves	91.7	46.9	6.3	8.4	84.7
	roots	96.4	75.6	31.4	21.1	25.0
Regina	stems	90.1	41.4	57.5	71.9	85.4
	leaves	100	79.4	27.7	39.5	36.9
	roots	97.6	93.0	91.0	39.2	61.1

[a] Recovery: following the PEG treatment, plants were subcultured in Hoagland's solution and maintained for 4 days.

DISCUSSION

The accumulation of WSC in response to environmental stresses is assumed to be associated with osmoregulation (Morgan, 1992) and/or with the protection of cellular membranes (Guy, 1990; Leprince et al., 1993). Both drought and salinity decrease the water potential of the external medium, and both can result in growth reduction, but the physiological mechanisms that mediates the response may be different in each case (Flowers and Yeo, 1986; Passioura, 1986; Erdei et al., 1990). Salt stress is composed of two components: salt toxicity and water stress.

Comparative data on the changes in sugar content in different drought- and salt-tolerant wheat seedlings under osmotic and salt stresses are scarce. The present results on the effect of drought and salinity on sugar accumulation in wheat seedlings demonstrate genotypical differences and provide details about the sugar components involved in drought and salt tolerance.

Sugars have been long known to increase in a wide range of plants grown at low moisture level (Martin et al., 1993; Rascio et al., 1994) and under salinity (Bolarin et al., 1995). Our data also confirm the fact that soluble sugar content seems to be a very sensitive and genotype-related marker for drought and salt tolerance improvement. The differences among the varieties in their ability to accumulate carbohydrates were evident not only under stress but also under control conditions as well (Figure 1). On this basis, Sakha, which possessed the highest sugar content in the shoot, can be defined as the most tolerant genotype, followed by Kobomugi, Chinese Spring, and Regina. This order is in agreement with the stress tolerance ability of these varieties presented under Materials and Methods.

Some authors (Al Hakimi et al., 1995; Kameli and Lösel, 1993) have reported that the accumulation of WSC under osmotic stress conditions appears to be one of the factors linked to stress tolerance, whereas others have found decreasing tendencies in sugar content (Hanson and Hitz, 1982) or constant values (Morgan, 1992). In the present experiments osmotic stress increased the WSC content in all varieties (Table 2). Furthermore, there was evidence that the effect of PEG and NaCl on sugar accumulation was different. Drought stress increased the WSC concentrations in all varieties and in all plant organs. These increases showed a positive correlation with tolerance. On the other hand, NaCl caused sugar accumulation only in the tolerant Sakha, whereas the sugar content decreased or changed only slightly in sensitive or moderately tolerant varieties. These typical changes were observed in stems and roots, whereas in all genotypes the sugar level decreased in leaves. Considering the components of the WSC, our results are in agreement with those of Kameli (1993), indicating that at least in these varieties glucose and fructose may play an important role during water stress, because their rates were clearly higher in stressed plants of the tolerant varieties and because their accumulation was the earliest response detected during the development of water stress (Figure 2). In tolerant genotypes glucose and fructose rose first (day 2) in all plant organs, whereas in sensitive ones this was true only for roots and stems. These results are in agreement with observations of Munns and Weir (1981) that the initial changes in osmotic potential were largely due to reducing sugars. On the other hand, Drossopoulos et al. (1987) found that sucrose was the only sugar showing a clear correlation with the degree of stress tolerance in old plants of wheat, whereas Timpa et al. (1986) found sucrose concentrations to be similar in stressed and well-watered cottons.

NaCl stress induced the inverse response: glucose, fructose, and sucrose rates increased sharply in all parts of the plant in sensitive genotypes but were unchanged (leaves and stems) or decreased (roots) in tolerant genotypes. This is probably due to the fact that lower salt tolerance may be related to the greater energetic cost of osmotic adjustment with sugar as opposed to Na or Cl, according to the low accumulation of saline ions and the high sugar accumulation. This hypothesis may explain the results reported by Bolarin (1995), who found higher sugar accumulations in salt-sensitive tomato seedlings than in tolerant ones.

Osmotic stress-induced fructan accumulation is well-known. Pilon-Smits et al. (1995) observed that transgenic tobacco plants that do not originally synthesize fructan accumulate bacterial fructan significantly better than wild types under PEG-mediated water stress. In the present experiments the concentrations of fructan increased under osmotic stress (Table 2). Among the plant organs studied, only leaves showed a positive correlation between fructan accumulation rate and the degree of drought tolerance of wheat varieties. In NaCl-treated plants, however, the fructan level in stems proved to be a good marker of salt tolerance, because a considerable accumulation was found in salt-tolerant genotypes, whereas a decrease was seen in sensitive ones (Figure 3). If a lower fructan content is the result of the salt treatment, then Kobomugi should be considered more sensitive to salt than Regina. This result is in agreement with the classification of cultivars of Nagy and Galiba (1995), based on the measurements of photosynthesis and abscisic acid concentration under similar experimental conditions. The different behavior of the two salt-sensitive varieties may be explained on the basis of their different drought tolerance. In the case of salt stress after drought, the "waste use of water" syndrome in drought-tolerant varieties may lead to an abrupt and large increase in water and possibly to an ion uptake. The effect of both Na^+ and Cl^- in excessive concentrations can be deleterious if a variety does not possess mechanisms such as Na/K discrimination (Shah et al., 1987) or the capability for the selective partitioning of Na^+, Cl^-, and K^+ ions (Greenway and Munns, 1980; Trivedi et al., 1991). Therefore, the preadaptation of the drought- and salt-sensitive variety to osmotic stress during drought may be beneficial under subsequent saline conditions (Regina), whereas the reverse tendency may be true for the drought-tolerant but salt-sensitive Kobomugi.

For a possible explanation of the differences found among the organs it is important to emphasize that fructan synthesis in leaves is dependent upon photosynthetic sucrose synthesis (SST), whereas in stems and roots, which are nonphotosynthetic tissues, fructan synthesis reflects translocated and subsequent metabolism. Under PEG treatment the highest fructan accumulation was observed in leaves, followed by stems and roots in a single plant, suggesting a PEG-induced fructan synthesis. NaCl dramatically decreased the fructan content in leaves, which was simultaneous to an increase in the rate of sucrose as an SST substrate, suggesting the NaCl inhibition of fructan synthesis. The high drought-induced fructan accumulation, despite the inhibiting effect of salinity, is in agreement with Hendry's (1993) opinion that the evolutionary significance of fructan was an adaptation to drought. Changes in the rate of sucrose, fructose, and fructan (Figure 2) as substrate and product were inversely based on the SST and FEH activity. In the control plants of all four varieties the DP of fructan did not exceed 9, in agreement with the findings of Blackbow et al. (1984) and Hendrix et al. (1986). The concentration of high DP fructan increased under both stress conditions. High DP fructan content appeared to be positively associated with freezing tolerance. A similar result was published by Suzuki and Nass (1988), who found that the rate of high DP fructan in tolerant wheat genotypes was higher than that in sensitive ones under cold hardiness. Furthermore, on the basis of the rapidity of accumulation of high DP fructan, leaves seem to be responsive to drought and roots to salinity.

In conclusion, the WSC content might be a useful trait in the selection of drought- and/or salt-tolerant wheat genotypes. Moreover, monosaccharides appear to play a central role in the initial response to both drought and salt stresses. The concentration of the principal component of WSC content increased in response to nonionic osmotic stress but mostly decreased due to ionic stress. From a comparison of the stress-induced sugar accumulation between tolerant and sensitive varieties, it appears that the most important factor does not seem to be sugar accumulation itself, but the earlier sugar accumulation in tolerant cultivars.

From the breeding point of view this study is a part of our project aimed to elucidate the role of the 5A chromosome in osmoregulation. If positive results are achieved in the identification of genes responsible for

drought- and salinity-induced sugar accumulation, the RFLP markers already available for the 5A chromosome can be used for marker-assisted selection in a breeding program. This program will eventually lead us to the identification of genotypes showing good sugar accumulating capability and presumably an increased level of drought and salt tolerance.

ABBREVIATIONS USED

Ch, Chinese Spring; glu, glucose; DP, degree of polymerization; fru, fructose; frn, fructan; gln, glucan; Ko, Kobomugi; PEG, poly(ethylene glycol); Re, Regina; Sa, Sakha; suc, sucrose; SD, standard deviation; TLC, thin-layer chromatography; WSC, total water-soluble carbohydrate.

LITERATURE CITED

Albrecht, G.; Kammerer, S.; Praznik, W. Fructan content of wheat seedlings (*Triticum aestivum* L.) under hypoxia and following reareation. *New Phytol.* **1993**, *123*, 471−476.

Al Hakimi, A.; Monneveux, P.; Galiba, G. Soluble sugars, proline and relative water content (RWC) as traits for improving drought tolerance and divergent selection for RWC from *T. polonicum* into *T. durum*. *J. Genet. Breed.* **1995**, *49*, 237−244.

Bancal, P.; Triboi, E. Temperature effects on fructan oligomer contents and fructan related enzyme activities in stems of wheat (*Triticum aestivum* L.) during grain filling. *New Phytol.* **1993**, *123*, 247−253.

Blackbow, W. M.; Darbyshire, B.; Pheloung, P. Fructan polymerised and depolymerised in the internodes of winter wheat as grain-filling progressed. *Plant Sci. Lett.* **1984**, *36*, 213−218.

Bolarin, M. C.; Santa-Cruz, A.; Cayuela, E.; Perez-Alfocea, F. Short-therm Changes in leaves and roots of cultivated and wild tomato seedling under salinity. *J. Plant Physiol.* **1995**, *147*, 463−468.

Colmer, T. D.; Epstein, E.; Dvorak, J. Differential solute regulation in leaf blades of various ages in salt-sensitive wheat and a salt-tolerant wheat x *Lophopyrum elongatum* (Host) A. Löve Amphiploid. *Plant Physiol.* **1995**, *108*, 1715−1724.

Drossopoulus, J. B.; Karamanos, A. J.; Niavis, C. A. Changes in ethanol soluble carbohydrates during the development of two wheat cultivars subjected to different degree of water stress. *Ann. Bot.* **1987**, *59*, 137−180.

Edelman, J.; Jefford, T. G. The metabolism of fructose polymers in plants. *Biochem. J.* **1964**, *93*, 148−161.

Erdei, L.; Trivedi, S.; Takeda, K.; Matsumoto, H. Effects of osmotic and salt stresses on the accumulation of polyamines in leaf segments from varieties differing in salt and drought tolerance. *J. Plant Physiol.* **1990**, *137*, 165−168.

Flowers, T. J.; Teo, A. R. Ion relations of plants under drought and salinity. *J. Plant Physiol.* **1986**, *13*, 75−91.

Galiba, G. In vitro adaptation for drought and cold hardiness in wheat. In *Plant Breeding Reviews*; Janik, J., Ed.; Wiley: New York, 1994; Vol. 12, pp 115−161.

Galiba, G.; Simoni-Sarkadi L.; Salgo, A.; Kocsy, G. Genotype dependent adaptation of wheat varieties to water stress in vitro. *J. Plant Physiol.* **1989**, *134*, 730−735.

Galiba, G.; Kocsy, G.; Kaur-Sawhney, R.; Sutka, J.; Galston, A. W. Chromosomal localisation of osmotic and salt stress-induced differential alteration in polyamine content in wheat. *Plant Sci.* **1993**, *92*, 203−211.

Galiba, G.; Kerepesi, I.; Snape, J. W.; Sutka, J. Location of a gene regulating cold-induced carbohydrate production on chromosomes 5A of wheat. *Theor. Appl. Genet.* **1997**, *95*, 265−270.

Greenway, H.; Munns R. Mechanism of salt tolerance in nonhalophytes. *Annu. Rev. Plant Physiol.* **1980**, *31*, 149−190.

Guy, C. L. Cold acclimation and freezing stress tolerance: role of protein metabolism. *Annu. Rev. Plant Phys. Plant Mol. Biol.* **1990**, *41*, 187−223.

Hanson, A. D.; Hitz, W. D. Metabolic responses of plant water deficit. *Annu. Rev. Plant Physiol.* **1982**, *33*, 163−203.

Hendrix, J. E.; Linden, J. C.; Smith, D. H.; Ross, C. W.; Park, I. K. Relationship of pre-anthesis fructan metabolism to grain number in winter wheat (*Triticum aestivum* L.). *Aust. J. Plant Physiol.* **1986**, *13*, 391−398.

Hendry, G. A. F.; Wallace, R. K. The origin, distribution, and evolutionary, significance of fructans. In *Science and Technology of Fructans*; Suzuki, M., Chatterton, N. J., Eds.; CRC Press: Boca Raton, FL, 1993; pp 119−139.

Housley, L.; Pollock, C. J. The metabolism of fructan in higher plants. In *Science and Technology of Fructans*; Suzuki, M., Chatterton, N. J., Eds.; CRC Press: Boca Raton, FL, 1993; pp 191−225.

Kameli, A.; Lösel, D. M. Carbohydrates and water status in wheat plants under water stress. *New Phytol.* **1993**, *125*, 609−614.

Kerepesi, I.; Toth, M.; Boross, L. Water-soluble carbohydrates in dried plant. *J. Agric. Food Chem.* **1996**, *44*, 3235−3239.

Leprince O.; Hendry, G. A. F.; McKersie, B. M. The mechanisms of desiccation tolerance in developing seeds. *Seed Sci. Res.* **1993**, *3*, 231−246.

Martin, M.; Miceli, F.; Morgan, J. A.; Scalet, M.; Zerbi, G. Synthesis of osmotically active substrates in winter wheat leaves as related to drought resistance of different genotypes. *J. Agric. Crop Sci.* **1993**, *171*, 176−184.

McKersie, B. D.; Leshen, Y. Y. *Stress and Stress Coping in Cultivated Plants*; Kluwer Academic Publishers: London, 1994; ISBN 0-7923-2827-2.

Morgan, J. M. Osmotic components and properties associated with genotypic differences in osmoregulation in wheat. *Aust. J. Plant Physiol.* **1992**, *19*, 67−76.

Munns, R. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. *Plant Cell Environ.* **1993**, *16*, 15−24.

Munns, R.; Weir, R. Contribution of sugars to osmotic adjustment in elongating and expanded zones of wheat leaves during moderate water deficit at two light levels. *Aust. J. Plant Physiol.* **1981**, *8*, 93−105.

Nagy, Z.; Galiba, G. Drought and salt tolerance are not necessarily linked: A study on wheat varieties differing in drought resistance under consecutive water and salinity stresses. *J. Plant Physiol.* **1995**, *145*, 168−174.

Passioura, J. B. Resistance to drought and salinity: Avenues for improvement. *Aust. J. Plant Phys.* **1986**, *13*, 191−201.

Pilon-Smits, E. A. H.; Ebskamp, M. J. M.; Paul, M. J.; Jeuken, M. J. W.; Weisbeek, P. J.; Smeekens, S. C. M. Improved performance of transgenic fructan-accumulating tobacco under drought stress. *Plant Physiol.* **1995**, *107*, 125−130.

Pollock, C. J.; Cairns, A. J. Fructan metabolism in grasses and cereals. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1991**, *42*, 77−101.

Rascio, A.; Planati, C.; Scalfati, G.; Tonti, A.; Fonzo, N. D. The accumulation of solutes and water binding streng in durum wheat. *Physiol. Planta.* **1994**, *90*, 715−721.

Rosa-Ibarra, M.; Maiti, R. K. Biochemical mechanism in glossy sorghum lines for resistance to salinity stress. *J. Plant Physiol.* **1995**, *146*, 515−519.

Santarius, K. A.; Bauer, J. Cryopreservation of spinach chloroplast membranes of low-molecular weight carbohydrates. I. Evidence for cryoprotection by a noncolligative-type mechanism. *Cryobiology* **1983**, *20*, 83−89.

Shah, S. H.; Gorham, J.; Foster, B. P.; Wyn Jones, R. G. Salt tolerance in the *Triticale*: the contribution of the d genomes to carbon selectivity in hexapoloid wheat. *J. Exp. Bot.* **1987**, *38*, 245−269.

Suzuki, M. Fructans in forage grasses with varying degrees of cold hardiness. *J. Plant Physiol.* **1989**, *134*, 224−231.

Suzuki, M.; Nass, H. G. Fructan in winter wheat, triticale and fall rye cultivars of varying cold hardiness. *J. Can. Bot.* **1988**, *66*, 1723−1728.

Timpa, J. D.; Burke, J. J.; Quinsenberry, J. E.; Wendt, C. W. Effect of water stress on the organic acid and carbohydrate content of wheat plant during the process of hardening for drought resistance. *Plant Physiol.* **1986**, *82*, 724−728.

Trivedi, S.; Galiba, G.; Sankhala, N.; Erdei, L. Responses to osmotic and NaCl stress of wheat varieties differing in drought and salt tolerance in callus cultures. *Plant Sci.* **1991**, *73*, 227−232.

Virgona, J. M.; Barlow, E. W. R. Drought stress induced changes in the non-structural carbohydrate composition of wheat stem. *Aust. J. Plant Physiol.* **1991**, *18*, 239−247.

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