Rapid *in vitro* screening of some salt tolerant bread wheats

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ABSTRACT.
A collection of 14 cultivars of *Triticum aestivum* was screened for tolerance to concentrations of 50, 100 and 150 mM NaCl. Excised mature embryos were *in vitro* cultured in Murashige and Skoog medium, supplemented with 30 g/L of sucrose and varying concentrations of salts. After 8 days, the cultured seedlings were evaluated for height, root length and root number. The test set showed tolerance to NaCl at concentrations of 50 mM and 100 mM. The cultivars were classified as tolerant (0-35% inhibited) and moderate tolerant (36-68% inhibited). In relation to height and root length, at 150 mM NaCl, cvs. Kharchia and Shorawaki were tolerant. NaCl did not affect root number significantly. Anions (in the presence of Na^+) and cations (in the presence of Cl^-) distinctly affected some parameters. For anions, either at low and at high concentrations, the ranking order of increasing inhibition was: $AcO^- = NO_2^- >> SO_4^= > HPO_4 > Cl^-$. For cations, the ranking order of increasing inhibition was: $Mn^{++} >> Ca^{++} > Mg^{++} = K^+ > Na^+$.

INDEX WORDS: cereal, embryo culture, growth, development, salinity, selection.

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INTRODUCTION.

Various wheat cultivars sustain growth and development under limited salt (NaCl) concentrations. The cultivars Chinese Spring, Kharchia and Shorawaki have been studied to characterize growth (Dvorák and Sosulski, 1974), induction of gene expression (Gulick and Dvorák, 1987), solute accumulation and distribution (Gorham *et al.*, 1986).
Traditionally, hydroculture has been used as a tool for salt tolerance screening (Gorham *et al.*, 1984). Recently, *in vitro* culture techniques have been succesfully introduced to generate cell cultures with salt tolerance (Maddock *et al.*, 1983) or to rescue developing embryos (Mujeeb-Kazi *et al.*, 1989). Mature wheat embryo culture offers another technique to evaluate growth and development (Díaz De León and Garibaldi-Meza, 1995) at different NaCl concentrations. In this study, the *in vitro* culture of mature embryos was used as a tool to confirm the salt tolerance trait of a set of wheat varieties from Egypt, India, México, Pakistan, China and U.S.A. as well as their response to the various ions encountered in salinity and needed for growth: Na^+, K^+, Mg^{++}, Ca^{++}, Mn^{++}, Cl^-, $SO_4^=$, HPO_4^-, NO_3^- and Acetate ion.

MATERIALS AND METHODS.

Plant material

A testor set of wheat varieties for salt tolerance including sensitive checks were provided by CIMMYT (International Maize and Wheat Improvement Center). Sakha 8 and Candeal, bread wheats, origined from Egypt and Mexico respectively; Kharchia, KRL1-4, PBW34 (durum), SNH-9 and WH157 origined from India; Lu26 S and Shorawaki origined from Pakistan; Chinese Spring originates from China; and N10714051, TC-181, TC-183 and TC-184 origined from U.S.A. The TC lines originated from a *in vitro* selection series for salt tolerance.

Embryo isolation and in vitro culture.

The procedures for seed sterilization and embryo isolation were carried out as described elsewhere (Díaz De León and Garibaldi-Meza, 1995). Excised embryos were placed in (non)modified Murashige and Skoog's medium (MS). Modified MS media contained different NaCl concentrations (0, 50, 100 and 150 mM). All media was solidified with 0.9%(w/v) agar, supplemented with 30 g/L sucrose and adjusted to pH 5.7 before autoclaving.

Plated embryos were cultured for 8 days under continuous illumination and at a temperature of 25°C. After 8 days, seedlings were taken out and evaluated for height, root lenght and root number.

Statistical analysis

Each experiment was run in triplicate (30 embryos comprised a replication). Data were weighed under ANOVA and MRA (Statgraphics) with an α=0.05.

Salt tolerance screening

Percentage of inhibition (%INH) was calculated as follows:

$$\% \text{ INH} = \frac{(\text{control value})-(\text{experimental value})}{\text{control value}} \times 100$$

Varieties based in % INH were classified as tolerant (T): T-1 (0-12%), T-2 (13-25%), T-3 (26-35%); moderate tolerant (MT): MT-1 (36-48%), MT-2 (49-61%), MT-3 (62-68%); and sensitive (S): S-1 (69-81%), S-2 (82-94%), S-3 (95-100%) in accordance with their response to the varying NaCl level.

RESULTS.

Response to salinity (NaCl)

Mature embryos, cultured in MS NaCl-free medium, developed seedlings with normal growth and development. After 8 days of culture, the height, root length and root number averaged 12.4 ± 1.6 and 5.1 ± 1.45 respectively, and 4.98 ± 0.54 roots (Tables 1). Mature embryos of the tested varieties showed distinct responses upon exposure to different levels of NaCl. At 50 mM, the height and root length of 7 cultivars were slightly inhibited in the T-1 and T-2 rank, while the rest remained unaffected (α=0.05) (Table 1). Lastly, the root number of the tested cultivars was unaffected (data not shown).

At 100 mM of NaCl, 6 varieties were inhibited in the T-3 rank with the exception of Shorawaki and Chinese Spring (T-1 rank); the rest had %INH values in the MT-1 and MT-2 ranks (Table 1). At 100 mM NaCl, 6 varieties were significatively (α=0.05) inhibited in root lenght (T rank) and the rest in the MT rank (Table 1). Again, 100 mM NaCl did not affect root number in the tested set (data not shown).
At 150 mM NaCl, cv. Shorawaki was tolerant; 3 cvs. were MT-3 while the rest were classified down to S-1 (Table 1). For root lenght, only PBW34 and Candeal got classified in the T rank while the rest kept in the MT rank (Table 1). Root number was not significantly (α=0.05) affected (data not shown).

Table 1. Effects of varying concentrations of NaCl on seedlings. The letters mean groups (α = 0.05) as weighed by ANOVA and MRA (statgraphics software).

	HEIGHT (cm)/ROOT LENGHT (cm)			
	NaCl (mM)			
T. aestivum	control	50	100	150
WH157	11.9a/4.8a	8.7b/3.3b	5.2c/2.4c	2.7d/0.9d
Kharchia	11.0a/2.1a	6.8b/3.0ab	7.1b/2.3ab	5.2b/1.5b
KRL 1-4	11.8a/5.4a	8.6b/3.6b	7.1b/2.4ab	3.5c/1.3d
SNH 9	10.5a/5.5a	8.4ab/3.7b	7.7b/3.0b	2.6c/1.7c
PBW34	10.5a/3.9a	10.5a/4.4a	7.3b/3.5a	2.4c/2.1b
Lu 26 S	12.0a/4.8a	9.8ab/4.7a	7.0bc/3.5b	4.4c/1.5c
Sakha 8	12.8a/4.2a	11.4a/4.3a	7.9b/2.7b	5.0c/1.9c
Shorawaki	11.2a/3.2a	11.7a/3.8a	8.9a/3.0b	9.2a/2.3c
Candeal	13.0a/5.4a	10.2b/3.0b	2.2c/2.9b	5.0c/1.7c
N1071405-1-5	13.8a/5.3a	8.9b/2.7b	9.0b/2.2b	7.1b/1.9b
Chinese Spring	15.3a/6.0a	13.7a/4.2b	3.2a/2.9c	7.6b/2.2c
TC-181	13.3a/4.4a	11.3b/5.8a	7.0c/5.8a	4.9d/5.4b
TC-183	12.5a/7.2a	8.7b/3.8b	7.5b/2.7c	4.9c/1.6d
TC-184	10.7a/7.6a	10.0a/4.0b	5.2b/2.2c	2.8c/2.1c

Effect of cations and anions
The cultivars PBW34, Kharchia and Shorawaki were tested for effects of different anions (sodium ion kept constant) and cations (chlorine ion kept constant). In these experiments the cultivars, under nonstress conditions, gave average values of 12.42 ± 1.41 cm, 4.17 ± 1.05 cm and 5.33 ± 0.42 for height, root length and root number respectively (Tables 2,3 and 4).

Table 2. Effects of cations and anions on seedling height. a= PBW34; b= Kharchia; c= Shorawaki. The letters mean groups (α = 0.05) as weighed by ANOVA and MRA (statgraphics software).

CONTROL (cm)				HEIGHT (cm) Na$^+$ salt (mM)					
				100			150		
a	b	c	ANION	a	b	c	a	b	c
10.5^{a}	11.0^{a}	11.2a	Cl^-	7.3b	7.1b	8.9a	2.4c	5.2b	9.2a
13.9^{a}	14.7^{a}	13.1a	HPO_4^-	3.9b	2.4b	4.0b	1.3c	0.3c	1.1c
9.4^{a}	11.4^{a}	11.3a	NO_3^-	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b
11.2^{a}	14.0^{a}	12.9a	AcO^-	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b
14.3^{a}	13.0^{a}	12.0a	$SO_4^=$	2.9b	4.6b	5.5b	0.8c	1.1c	2.6c
			CATION			Cl^- salt (mM)			
10.5^{a}	11.0^{a}	11.2a	Na^+	7.3b	7.1b	8.9a	2.4c	5.2b	9.2a
12.3^{a}	15.3^{a}	14.4a	K^+	6.7b	7.0b	12.7a	5.2b	6.3b	8.7b
11.0^{a}	13.6^{a}	13.8a	Mg^{2+}	5.6b	12.2^{a}	11.0a	5.5b	6.2b	12.0a
12.4^{a}	11.0^{a}	13.1a	Ca^{2+}	5.7b	3.2b	4.3b	1.1c	0.4c	0.4c
12.0^{a}	14.8^{a}	12.0a	Mn^{2+}	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b

Table 3. Effects of cations and anions on root length. a= PBW34; b= Kharchia; c= Shorawaki. The letters mean groups (α = 0.05) as weighed by ANOVA and MRA (statgraphics software).

CONTROL (cm)				ROOT LENGHT (cm) Na$^+$ salt (mM)					
				100			150		
a	b	c	ANION	a	b	c	a	b	c
3.9a	2.1^{ab}	3.2ab	Cl^-	3.6a	2.8^{ab}	3.0b	2.1b	1.5b	2.3c
3.9a	6.8a	4.8a	HPO_4^-	1.5b	2.1b	2.7b	0.7c	0.7c	0.8c
3.5a	3.9a	2.9a	NO_3^-	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b
4.2a	4.0a	3.8a	AcO^-	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b
3.9a	4.8a	5.9a	$SO_4^=$	2.4b	1.9b	2.3b	0.7c	0.9c	0.9c
			CATION			Cl^- salt (mM)			
3.9a	11.0^{a}	11.2a	Na^+	3.6a	2.8^{ab}	3.0b	2.1b	1.5b	2.3c
5.5a	3.9a	4.3a	K^+	3.5b	2.1b	2.6b	2.3c	1.3c	1.8c
5.6a	3.6a	3.6a	Mg^{2+}	2.3b	2.3b	2.1b	1.0c	1.4c	3.1a
4.5a	5.1a	3.4a	Ca^{2+}	2.0b	1.1b	0.9b	0.5c	0.2b	0.2c
5.2a	3.6a	4.1a	Mn^{2+}	0.1b	0.2b	0.3b	0.0b	0.0b	0.0b

Table 4. Effects of cations and anions on root number. a= PBW34; b= Kharchia; c= Shorawaki. The letters mean groups (α = 0.05) as weighed by ANOVA and MRA (statgraphics software).

CONTROL (cm)				ROOT NUMBER (#) Na⁺ salt (mM)					
				100			150		
a	b	c	ANION	a	b	c	a	b	c
5.2a	6.1a	4.6a	Cl^-	5.3a	4.6a	5.2a	4.3b	4.6a	5.6a
5.5a	5.3a	4.9b	HPO_4^-	4.5a	5.8a	6.4a	3.2b	2.4b	3.0b
5.4a	4.9a	5.4a	NO_3^-	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b
5.7a	6.0a	5.2a	AcO^-	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b
5.9a	5.1a	5.0a	$SO_4^=$	3.9b	4.9a	4.4ab	2.1c	3.5b	3.8b
			CATION	Cl^- salt (mM)					
5.2a	6.1a	4.6a	Na^+	5.3a	4.6a	5.2a	4.3b	4.6a	5.6a
5.8a	5.8a	5.3b	K^+	4.9ab	5.5a	7.3a	4.6b	4.9a	6.0b
5.6a	5.6^{ab}	5.3a	Mg^{2+}	4.4b	6.1a	6.0a	4.4b	4.4b	5.9a
5.8a	4.4a	5.0a	Ca^{2+}	4.4b	3.3b	3.8b	1.6c	0.8c	1.2c
6.0a	6.3a	5.7a	Mn^{2+}	0.4b	0.9b	0.8b	0.0c	0.0c	0.0c

Height

Anions. At 100 mM anion concentration (Na^+ kept constant), the increasing inhibitory rank order was: NO_3^-= AcO^- > HPO_4^-= $SO_4^=$ > Cl^-(Table 3). The varieties expressed tolerance to chloride ions. The cultivars were sensitive to sodium salts when the accompanying anions were HPO_4^-, NO_3^- and AcO^- Kharchia and Shorawaki were MT-3 to $SO_4^=$(Table 2). At a higher concentration (150 mM), all anions inhibited seedling height in S-3 rank; cvs. Kharchia 65 and Shorawaki classified into the T rank against chlorine ions only (Table 2).

Cations. At 100 mM of cation concentration (Cl^- kept constant), a general increasing rank order was: Mn^{2+} > Ca^{2+} > K^+ > Na^+. All varieties showed sensitivity to Mn^{2+} (Table 2). However, the variety Shorawaki was distinguished by its tolerance to Na^+, K^+ and Mg^{2+} (Table 2). At 150 mM concentration the cv. Shorawaki asserted its tolerance to cations Na^+ and Mg^{2+} and Kharchia only to Na^+ (Table 2).

Root length

Anions. At 100 mM anion concentration (Na^+ kept constant) tolerance for root length was similar to height. The varieties classified as T to chloride ions and MT to $SO_4^=$ ions (Table 3). At 150 mM, cv. Kharchia showed a better chloride tolerance than the other varieties (Table 3).

Cations. The effects of cations at both concentrations resembled those described for height (Table 3).

Root number

Anions. At 100 mM anion concentration (Na^+ kept constant) the cultivars were tolerant except for NO_3^- and AcO^- (Table 4); HPO_4^- enhanced root proliferation of Shorawaki. A concentration of 150 mM did not change the pattern of varieties except for the degree of tolerance (Table 4).

Cations. At 100 mM of cation concentration (Cl^- kept constant) all cultivars expressed tolerance except for Mn^{2+} (Table 4). At 150 mM concentration the same pattern was observed for Na^+, K^+ and Mg^{2+}. All varieties were sensitive to Mn^{2+} and Ca^{2+} (Table 4).

DISCUSSION.

Wheat cultivars expressed tolerance to NaCl at 50 and 100 mM, while at 150 mM PBW34, WH157 and TC-184 were affected by NaCl. The *in vitro* culture of mature embryos confirmed early results obtained in hydroponic experiments about the salinity tolerance of Chinese Spring, Kharchia and Shorawaki (Gorham *et al.*, 1986). The *in vitro* screening of cv. Shorawaki showed a superior performance to salt tolerance of all tested cultivars; in hydroculture its biomass and K/Na discrimination ratio had superseeded that of Kharchia and Lu 26 S. Additionaly, cv. Shorawaki showed tolerance to cations different from Na^+, resembling the amphiploid of *T. aestivum* x *Elytrigia elongata* trait (Dvorák and Roos, 1986), except for sensitivity to Mn^{2+} and Ca^{2+}. In our study Shorawaki and Karchia were sensitive to anions of NO_3^-, AcO^- and $SO_4^=$. Additionaly Shorawaki performed better than Chinese Spring, compared with the results from hydroponic culture (Dvorák and Roos, 1986).

At 100 mM concentration, HPO_4^- and K^+ were better enhancers of root proliferation when accompanied by Na^+ and Cl^- respectively, for cv. Shorawaki (Table 4). This phenomenon was remarkably expressed in Shorawaki.

Finally, as a result of this study the testor set now includes cvs. Kharchia, Lu 26 S, Shorawaki, N1071405-1-5 as a NaCl tolerant (T). The moderate tolerant (MT) germplasm would be KRL 1-4, SNH 9, Sakha 8, Candeal, Chinese Spring, TC-181, TC-183 and TC-184.

It is apparent that *in vitro* culture of mature embryos is a useful tool, which complements hydroculture and field evaluations. Though some variations exist in classifying germplasm in different cultivar categories for tolerance between our findings and those of Gorham *et al.*, 1986 and Trivadi *et al.*, 1991, the response which qualifies an entry as being tolerant or medium-tolerant seems to be an adequate screening index for breeding tolerant wheat cultivars. Some variation may be attributed to the techniques having used which have incorporated hydroponics, *in vitro* screening and response of callus cultures to osmotic conditions. The influence of factors may also prove to be widely crucial in selection of salt tolerant varieties as suggested by Trivedi *et al.*, 1991.

The separation of Chinese Spring, KRL 1-4 and Sakha-8 from the tolerant set to a medium tolerant set may be a consequence of such evaluation procedures. The derived KRL 1-4 cultivar from Kharchia genetically would be expected to have a T category but its MT classification still keeps it as a potent source for utilization for wheat production in saline/sodic areas. Our initial *in vitro* testing is aimed to provide a rapid homogenous media screen around which advanced complex procedures can be developed leading to an ultimate field correlation salinity tolerant response of categorized cultivars.

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