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NaCl effects in *Zea mays* L. x *Tripsacum dactyloides* (L.) L. hybrid calli and plants

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Abbreviations: 2,4-D: 2,4-dichlorophenoxyacetic acid
MT: *Zea mays* L. (2n = 40) and *Tripsacum dactyloides* (2n = 72) hybrid

High salt concentrations in soils negatively affect maize growth. Techniques such as remote hybridization and *in vitro* selection have been extensively used to accelerate breeding processes. In order to determine the usefulness of *Tripsacum* to improve salt tolerance in maize, the effects of NaCl, *in vitro* and *in vivo*, were evaluated in an intergeneric hybrid (MT) obtained from crossing *Zea mays* with *Tripsacum dactyloides*. Organogenic calli, induced from immature MT hybrid embryos, were exposed to different NaCl concentrations and the survival and regeneration percentages were calculated. Plants of the MT hybrid, obtained from the organogenic calli, were exposed to NaCl concentrations considered harmful for maize. The shoot dry weights of plants exposed to 250 mM NaCl did not show significant differences respect to the control ones. Although sodium content in shoots was incremented 2,5 fold, it was not toxic for this material. The MT hybrid showed better behavior, *in vitro* and *in vivo*, that maize genotypes exposed to similar conditions.

High salt concentrations in soils negatively affect maize growth and, consequently, produce a large drop in yield (Pasternak et al. 1995). In many countries of the world soil salinity is a serious problem for agriculture and,

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consequently, the development of salinity tolerant genotypes is considered an important research subject for genetic improvement (Flowers et al. 1997).

Salinity causes both, hyperionic and hyperosmotic stress effects, and the consequence of these can be plant demise. Most common stress is caused by high Na^+ and Cl^- concentrations in soil solution (Hasegawa et al. 2000). Maize soil salinity effects have been widely studied. On one hand, NaCl presence in soil solution affects crop water relations, which becomes in an osmotic stress for maize plants (Cramer et al. 1994; Hasegawa et al. 2000). And on the other hand, shoot Na^+ concentration in maize increases with NaCl increments in soil solution, which involves ionic balance alterations (Shabala et al. 1998).

Resistance to abiotic stresses in general, and to salt stress in particular is under polygenic control, which have hindered the improvement in this aspect (Flowers and Yeo, 1995; Winicov, 1996). Techniques such as remote hybridization and *in vitro* selection have been greatly used to accelerate the breeding process. Hybridization of two phylogenetically distant species offers a great potential to increase the genetic variability, whether by introgression of desirable characters in the cultivated species (introduction of simple genes or addition/substitution/translocation of chromosomes or chromosome segments), or by the generation of new allopolyploids with one or more genomes and several useful characteristics of both parental species (Matzk, 1997).

Table 1. Survival and regeneration percentages of MT hybrid calli exposed to different NaCl concentrations during 35 days.

	NaCl (mM)			
	0	70	140	210
Regeneration	61.5 a	40 b	30 c	0 d
Survival	92.3 a	60 b	40 c	8.3 d

Different letters in a row indicate significant differences between the means (Tuckey, α: 0.05).

Tripsacum dactyloides is a highly palatable and productive perennial grass (Faix et al. 1980), which shows tolerance to different environmental stresses (Foy, 1997; Clark et al. 1998; Ray et al. 1999). Long-term organogenic calli have been obtained from tetraploid maize (2n = 40) x *Tripsacum dactyloides* (2n = 72) hybrid embryos. Regenerated hybrid plants showed a somatic chromosome number 2n = 56 (García et al. 2000) and high tolerance to salinity (Pesqueira et al. 2003) and low temperatures (Jatimliansky et al. 2004). Even though maize x *T. dactyloides* F1 hybrid plants exhibit very low fertility, a few viable seeds have been obtained (Leblanc et al. 1995; Sokolov et al. 2000; Molina et al. 2005). Fertility and seed production increased in subsequent generations by reducing the number of *Tripsacum* chromosomes (Khatypova et al. 2002; Molina et al. 2005). Further, meiotic cells of these hybrids showed maize and *Tripsacum* chromosomes pairing, which suggest the possibility of genetic recombination between parental species (Molina et al. 2005). In this context, maize x *T. dactyloides* F1 hybrids could be a source of salinity tolerance to use in a maize improvement program.

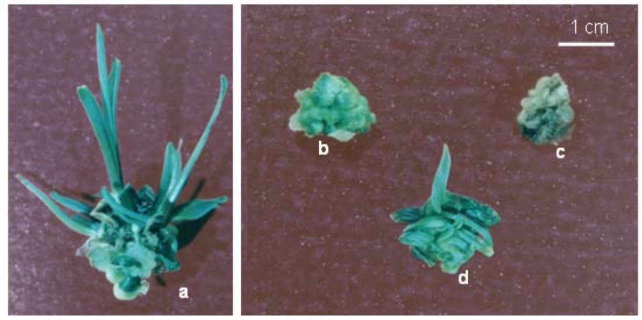

Figure 1. MT hybrid caulogenic calli 35 days after *in vitro* culture: (a) Shoot elongation in the basic medium with 2,4-D; (b-c-d) Calli exposed to different NaCl concentrations; (b) Caulogenesis inhibition; (c) Necrosis; (d)- Caulogenesis (at least one shoot).

Although methods such as remote hybridization and *in vitro* selection have been widely used to accelerate plant breeding process, some frequent limitations observed have been the difficulty to regenerate plants from the selected material (Lutts et al. 1999) or the lack of correlation between *in vitro* and *in vivo* tolerance (McCoy, 1987).

The aims of the present study were to evaluate the effects of different NaCl concentrations on the following parameters of the MT hybrid: i) regeneration capacity and survival of organogenic calli; ii) growth of the regenerated plants exposed to a NaCl concentration considered to be harmful to cultivated maize; iii) plant shoot concentrations of sodium, potassium and calcium.

MATERIALS AND METHODS

Plant Material

MT hybrid organogenic calli, obtained from crossing the maize line N107B (2n = 40) and *Tripsacum dactyloides* (2n = 72); and MT hybrid plants, obtained from regenerated shoots.

In vitro culture and plant regeneration

MT hybrid calli growth and shoot regeneration, were carried out in a basic medium (García et al. 1992) with 1 mg L^{-1} 2,4-D. The regenerated shoots were separated and individually transferred to the basic medium to induce root production. After 45 days, the plantlets were transplanted into 200 ml pots with a mixture of equal parts of earthworm compost and sterile soil, and covered with plastic bags. After 50 days rusticated plants were transferred to bigger pots (1700 ml) and watered with tap water.

NaCl treatments *in vitro*

MT hybrid calli were exposed to the following NaCl concentrations: 0; 70; 140 and 210 mM. After 35 days *in vitro*, in a growth room with 13/11 h photoperiod and 30/25ºC and a photon flux density of 31 µmol m^{-2} s^{-1}, percentages of living (green callus) and regenerating callus (those showing at least one shoot) were determined.

In vivo methodology

Sixty plants with an average height of 45 cm, were randomly selected. Half of the plants were watered during 21 days with 250 mM NaCl, and the other ones with tap water (control). The experiment was carried out in the greenhouse. When the experiment was finished, fresh and dry weights of the shoot, as well as the root were registered. Dried and milled shoots were digested with nitric and percloric acids. Na^+, Ca^{2+} and K^+ contents were determined by atomic absorption spectrophotometry (Isaac and Kerber, 1971).

Table 2. Shoot and root fresh and dry weights of MT hybrid plants watered with 250 mM NaCl and with tap water during 20 days.

	Control	250 mM NaCl
Substrate		
E.C. (dS m^{-1})	2.4	8.4
pH	6.9	7.1
Shoot (S)		
Fresh weight (g pl^{-1})	5.20 a	6.18 a
Dry weight (g pl^{-1})	0.98 a	1.44 a
Root (R)		
Dry weight	0.14 a	0.55 b
S:R	7.0	2.6

Different letters in a row indicate significant differences between the means (LSD, α: 0.05).

RESULTS AND DISCUSSION

NaCl effects on MT hybrid organogenic calli

MT hybrid calli survival and regeneration decreased as NaCl concentration increased in the culture medium (Figure 1). After 35 days in culture, 40% of the calli exposed to 140 mM NaCl survive and 30% of these showed shoot regeneration (Table 1). Regenerated and rusticated plants were phenotypically normal and homogeneous, contrary to what have been observed in rice, for example, a total inhibition of shoot regeneration in presence of 100 mM NaCl (Lutts et al. 1999).

NaCl effects on MT hybrid plants

MT hybrid plants watered with 250 mM NaCl did not show significant differences, neither in the fresh nor in the dry shoot weights, compared to control plants. However, root dry weight was significantly higher in NaCl treated plants than in the control ones (Table 2). This increment in root dry weight, determined a shoot/root rate 2.7 fold less than control plants. A drought tolerant maize hybrid, under drought conditions, produced a similar effect, suggesting an osmotic response (Grzesiak et al.1999). Nevertheless, it has been shown that NaCl ionic effect was responsible for the root growth rate increment observed in tomato transformed roots (Talano et al. 2002).

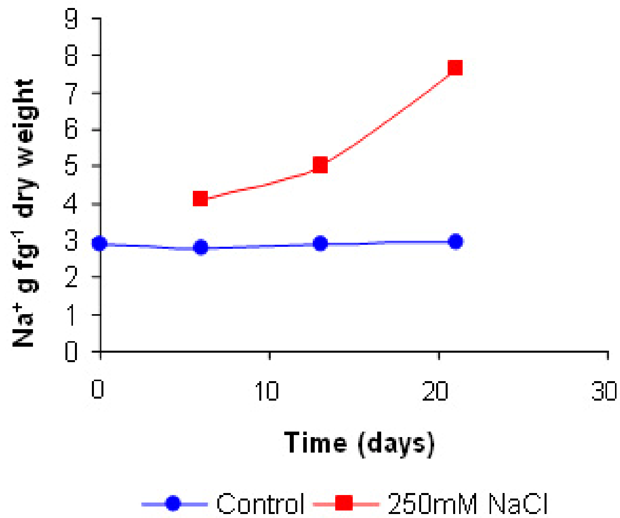

Figure 2. Sodium content evolution in shoots of MT hybrid plants watered with 250 mM NaCl and with tap water during 20 days.

Sodium content in the MT hybrid plants significantly increased respect to the control, when they were watered with 170 mM NaCl (2.7 $g.kg^{-1}$ *vs.* 6.8 $g.kg^{-1}$, control and stress, respectively) (Pesqueira et al. 2002) as well as with 250 mM NaCl (2.94 $g.kg^{-1}$ *vs.* 7.65 $g.kg^{-1}$ control and stress, respectively) (Figure 2). Meanwhile calcium and potassium concentrations did not result statistically different (Table 3). Cramer et al. (1994) observed sodium accumulation in maize salinity tolerant cultivars exposed to NaCl, which suggests that the principal cause in salt sensitivity is the osmotic effect.

Table 3. Sodium, calcium and potassium shoot concentration of MT hybrid plants watered with 250 mM NaCl and with tap water during 20 days.

Treatment	Shoot concentration (g kg^{-1} dry weight)		
	Ca^{+2}	Na^+	K^+
Control	3.75a	2.94a	31.4a
250 mM NaCl	3.70a	7.65b	30.9a

Different letters in a column indicate significant differences between the means (LSD, α: 0,01).

In conclusion, our results and those previously reported, suggest that MT hybrid salt tolerance is based, firstly on the strategy to accumulate sodium, and consequently to lower leaves water potential, maintaining the turgor pressure required for vegetative growth; and secondly, on the capacity to lower shoot/root rate, which is a favourable aspect for plant water balance. Compared to the controls, MT hybrid plants watered with 250 mM NaCl, did not show significant differences neither in the dry weights nor in the calcium and potassium concentrations. In addition, although sodium content in shoots increased 2.5 fold, when watered with NaCl solution, it was not toxic for this genotype. Therefore, the MT hybrid showed better behavior, *in vitro* as well as *in vivo*, that maize genotypes exposed to similar conditions. It deserves further research to determine the presence of any biochemical marker of salt stress tolerance, which may be participating in the observed response.

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