NaCl tolerance in maize (Zea mays ssp. mays) x Tripsacum dactyloides L. hybrid calli and regenerated plants

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Abstract

Since corn has been classified as one of the most sensitive crops to soil salinity, the evaluation of NaCl tolerance of a hybrid of Zea mays ssp. mays (2n=40) and Tripsacum dactyloides (2n=72), was considered of interest to determine the possibility of using this germplasm in corn tolerance improvement. Long-term organogenic calli, obtained from immature hybrid embryos, have shown high rates of multiplication and plant regeneration. After in vitro treatment of these calli with different levels of salinity, the survival and regeneration percentages and the daily relative weight increments were calculated. Regenerated plants were transplanted to pots and watered with NaCl solution (6.1 dS m⁻¹) in order to assess tolerance at a whole plant level. Measurements of height, number of leaves per plant, and fresh and dry weights were taken. Exposure to 170 mM NaCl in vitro during 35 days and in vivo during 20 days produced a fresh weight decrease of 51% in calli and 31% in plants, respectively. These results indicate an improved tolerance of the maize/Tripsacum hybrid to salinity stress, in vitro and in vivo, compared with results from previous reports using other corn genotypes under similar conditions.

Key words: intergeneric hybrid, salinity stress, organogenic callus, corn, gamagrass.

Resumen

Tolerancia a NaCl en callos y plantas regeneradas de un híbrido de maíz (Zea mays ssp. mays) x Tripsacum dactyloides L.

Dado que el maíz ha sido clasificado como uno de los cultivos más sensibles a la salinidad en el suelo, se consideró interesante evaluar la respuesta a la salinidad causada por NaCl in vitro e in vivo de un híbrido entre un maíz tetraploide (Zea mays ssp. mays, 2n=40) y Tripsacum dactyloides (2n=72), con el fin de determinar la utilidad de este material como base de partida para mejorar la tolerancia del maíz. A partir del rescate de embriones inmaduros se obtuvieron callos organogénicos que mantuvieron una alta tasa de multiplicación y regeneración de plantas por largos periodos. Dichos callos se sometieron a distintos niveles de salinidad, y se calcularon los porcentajes de supervivencia y regeneración, así como el incremento en peso fresco relativo diario. A partir de callos organogénicos del mismo híbrido, se obtuvieron plantas que se transplantaron a macetas y se regaron con solución salina (6,1 dS m⁻¹), a fin de evaluar la tolerancia in vivo. Se midieron las variables altura de planta, número de hojas por planta, peso fresco y peso seco. La exposición a 170 mM de NaCl durante 35 días in vitro y 20 días in vivo, produjo una disminución del peso fresco del 51% en callos y del 31% en plantas, respectivamente. Los resultados obtenidos muestran una mayor tolerancia del híbrido, tanto in vitro como in vivo, respecto a distintos genotipos de maíz cultivados en condiciones similares.

Palabras clave: híbrido intergenérico, estrés salino, callo organogénico, maíz.

Introduction

High salt concentrations in soils negatively affect corn growth and, consequently, produce a large drop in yield (Ashraf and McNeilley, 1989; Pasternak et al., 1995). Although Maas and Hoffman (1977) classified corn among the crops most sensitive to soil salinity, in related reports a wide phenotypic variation was observed in response to this stress (Ashraf and McNeilley, 1989; Epstein, 1985).
The effects of saline stress on corn have been well studied. Firstly, it has been shown to affect water relations (Cramer, 1992), which generates plant osmotic stress (Cramer et al., 1994). Recently, it has been found that the presence of salt not only reduces the available water in the soil but also affects the permeability of root cortex, by altering cell membrane water channels (Hasegawa et al., 2000).

Other corn plant physiological parameters affected by soil salinity include cell wall rheological properties (Cramer, 1992; Pimentel, 1999), protein synthesis (Ramagopal, 1986; Yang et al., 1995; Saneoka et al., 1995), caryopsis soluble carbohydrate concentration (Pasternak et al., 1995), leaf bioelectrical activity (Shabala et al., 1998), and ionic balance (Hassan et al., 1970; Ashraf and McNeill, 1989; Shabala et al., 1998). However, there was no consistent correlation between any of these parameters and salinity tolerance, measured as vegetative growth or yield. Therefore, the change in plant growth or yield compared with a control is the most reliable indicator of the tolerance to saline stress (Cramer et al., 1994).

Hybridization of two phylogenetically distant species offers a great potential to increase the genetic variability, whether by the introgression of desirable characters in the cultivated species (introduction of simple genes or the 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. The Maydeae tribe includes the genera Zea, Tripsacum (in America) and Coix (in India). Tripsacum has interesting characteristics to be used in crossings to generate genetic variability in corn. It is a complex genus that includes 16 species (Li et al., 1999; Kindiger and Dewald, 1997) all of which are perennial but with different morphology and adapted to different environmental conditions (Wilkes, 1979). Although Zea and Tripsacum are reproductively isolated by gametophytic and sporophytic barriers, fecundation occurs cutting corn stigmas before hand pollination (Mangelsdorf and Reeves, 1931). Hybrid plants can be obtained by immature embryo rescue (James, 1979; Bernard and Jewell, 1985) and induction of somatic embryogenesis (Furini and Jewell, 1995; Garcia and Molina, 1997).

Long-term organogenic calli were induced from immature embryos obtained from crosses between tetraploid maize and T. dactyloides (Garcia and Molina, 1997). Several authors have observed that chromosomal stability in vitro is affected by factors such as the explant, the genotype and, mainly, the culture age (McCoy and Phillips, 1982; Molina and Garcia, 1998). Therefore, long-term organogenic calli could be useful to induce chromosomal alterations that increase the exchange frequency between the chromosomes of the parental species. Moreover, induction of saline stress in long-term organogenic callus cultures and, especially during the in vitro regeneration process, is a convenient way of selecting tolerant genotypes (Johnson et al., 1991). However, little is known about the response of maize x Tripsacum hybrids to saline stress, which is essential to implement an improvement programme.

The selection of tolerant genotypes in vitro opens up the possibility of accelerating the improvement process, but there are some restrictions such as the difficulty of regenerating plants from the selected material (McCoy, 1987a; Lutts et al., 1999), and the occasional lack of correlation between tolerance in vitro and the response of the whole plant in vivo (McCoy, 1987b). Although the comparative effect of NaCl and mannitol on protein synthesis has been studied in corn (Ramagopal, 1986), there are no published data of the effects of saline stress on plant regeneration in vitro, or on the correlation between in vitro and in vivo tolerance, factors that would be essential to assess new genetic combinations obtained from embryo rescue.

The objectives of this work were: i) to determine the in vitro effects of NaCl stress on fresh weight increase, regeneration capacity and survival of organogenic calli of the hybrid Zea mays ssp. mays (2n=40) x Tripsacum dactyloides L. (2n=72), and ii) to evaluate the growth of the hybrid regenerated plants exposed to 170 mM NaCl, a saline concentration considered to be harmful to cultivated maize.

**Material and methods**

Maize x Tripsacum (N107BxT) hybrid callus and plants were obtained as follow: the tetraploid maize line N107B (2n=40, supplied by the Corn Genetic Coop. Stock Center, Missouri, U.S.) was hand pollinated with Tripsacum dactyloides (2n=72), and 12 days old immature embryos were isolated and cultured in presence of 4 µM 2,4-dichlorophenoxyacetic acid (2,4-D) (Garcia and Molina, 1997).

Maize x Tripsacum organogenic calli were grown in vitro for three years by monthly subcultures to the basic medium of Garcia et al. (1992) supplemented
with 4 μM 2,4-D. On average, 11 calli of approximately 200 mg each, were exposed to each salinity concentration (0, 70, 140, 170, 210 and 250 mM NaCl). The experiment was repeated four times. The initial weight (Wi) of each callus was recorded just before the beginning of treatments and the final weight (Wf) was recorded after 35 days of growth at 30°C, with 13 h of photoperiod. The daily increase in relative weight (RWI) was calculated using the following formula: 
\[ RWI = \frac{(W_f - W_i)}{W_i} \times d^{-1} \]
The survival and regeneration percentages were calculated by considering green calli as live ones and calli with regeneration capacity as those with at least one shoot.

Regenerated shoots were subcultured to a basic medium without plant growth regulators to induce rooting. 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. In the first watering, Benomyl (1 g L^{-1}) was used as a systemic fungicide. When the plants reached an average height of 30 cm they were transplanted to 1700 ml pots with soil and irrigated with tap water for seven days. On the eighth day, 50 plants were randomly selected, of which 25 were watered for 20 days with saline solution (170 mM NaCl; 6.1 dS m^{-1}) and the rest with tap water (control; 1.5 dS m^{-1}). Both groups were watered every two days with 100 ml per pot. The experiment was done in a greenhouse. Three, eight, fifteen and twenty days after the first watering, four plants were randomly selected for evaluation, the height was measured, the number of leaves per plant was counted and the shoot was cut to determine their fresh weight (FW) and dry weight (DW) after heating at 70°C for three days.

**Statistical analysis**

The results were analysed with the statistical software Statistix for Windows, Copyright 1985. More specifically, variance analysis, Tukey’s Least Significant Differences (LSD), determination coefficient (linear regression) and F test for comparison of means were applied.

**Results**

Survival and regeneration of the hybrid N107BxT organogenic calli declined as the medium saline concentration increased (R²=0.99 and R²=0.96, respectively). However, an increase of 50% in NaCl concentration (from 70 mM to 140 mM) only produced a drop of 15% in plant regeneration (Table 1).

Callus relative daily weight increment (RWI d^{-1}) was strongly affected by the saline concentration (R²=0.95). Tukey’s LSD (5%) separated treatments into four homogeneous groups, with no significant differences between the means within the groups (Table 1).

At the whole plant level, the increase in fresh and dry weight per plant declined, compared to control plants (0 mM NaCl), when watered with a 170 mM NaCl solution (Figure 1). Assuming a normal distribution with a significance level of 0.05, the significant differences between fresh weights, of plants watered with NaCl and control plants, appeared 15 days after the first watering (Figure 1A). However, there were no significant differences in dry weights (α: 0.05) between treated and control plants, during the 20 days of the experiment (Figure 1B).

Plant water content evolution clearly decreased in NaCl-treated plants compared to control ones (Table 2), which explains why significant differences were detected in the increases in plant fresh weight and not in dry weight.

**Discussion**

Organogenic calli of the maize/Tripsacum hybrid studied in this work, exhibited a 51% fresh weight re-

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**Table 1. Regeneration and survival percentages, and relative daily weight increase of hybrid N107BxT calli exposed to different NaCl concentrations**

<table>
<thead>
<tr>
<th>NaCl concentration (mM)</th>
<th>0</th>
<th>70</th>
<th>140</th>
<th>170</th>
<th>210</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regeneration (%)</td>
<td>61.5 a</td>
<td>40 b</td>
<td>30 c</td>
<td>—</td>
<td>0 d</td>
<td>—</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>92.3 a</td>
<td>60 b</td>
<td>40 c</td>
<td>—</td>
<td>8.3 d</td>
<td>—</td>
</tr>
<tr>
<td>RWI/day (g g^{-1} d^{-1})</td>
<td>0.123 a</td>
<td>0.087 b</td>
<td>0.071 bc</td>
<td>0.057 cd</td>
<td>0.059 bcd</td>
<td>0.033 d</td>
</tr>
</tbody>
</table>

Different letters in a row indicate significant differences between the means (Tukey, α: 0.05).
duction relative to the control when cultured in 170 mM NaCl during one month; on the other hand, Black Mexican Sweet corn, grown with 100 mM NaCl over the same period, showed a reduced growth relative to the control of 81.54% (Ramagopal, 1986), which indicates a markedly greater tolerance of the hybrid in vitro.

The medium NaCl concentration is a key factor to select salinity tolerance in vitro; for that reason, the best relationship between a maximum selection pressure and the regeneration rate of plants must be determined. Although there are no reports about the effects of NaCl on plant regeneration from maize callus, in rice, for example, a 100 mM NaCl concentration completely inhibited this process (Lutts et al., 1999). However, 30% of N107BxT calli maintained their regeneration capacity even in presence of 140 mM NaCl (Table 1). Regenerated plants were normal and phenotypically homogeneous, in contrast to the results of McCoy (1987a) who obtained abnormal plants with 45% dwarfism, from Medicago sativa cell lines exposed to NaCl.

Ashraf and McNeilly (1989) compared the response of nine corn cultivars watered with 180 mM NaCl for 30 days, and they observed that only five cultivars kept growing, with a maximum of 18%, of both fresh and dry final weight compared to the controls, in Sultan cultivar. Furthermore, the relative fresh and dry weights of Sultan plants (about 45% of control plants) in presence of 120 mM NaCl were still lower than those of the N107BxT hybrid cultured with 170 mM NaCl (69% and 84% of the control plants, respectively).

Cramer (1992) studied the responses of two corn cultivars with a different capacity to exclude sodium, in an attempt to determine which mechanisms were involved in the inhibition of growth by salinity, and concluded that the main cause of reduced corn growth in the presence of NaCl was osmotic stress. The results obtained for the hybrid in this work indicate that salinity also negatively affects water absorption. However, within the NaCl concentrations studied, the hybrid was able to maintain an increase in dry matter that did not significantly differ from the control, in spite of the less water content, suggesting the presence of some mechanisms that confer it the observed salinity tolerance.

The occasional lack of correlation between tolerance in vitro and the response of the whole plant is an important limitation to take into account when selecting tolerant genotypes in vitro. For example, in genus Medicago, almost all the genotypes which showed tolerance in vitro were sensitive to salinity at a whole plant level, and the opposite occurred in Medicago maritima, which showed salinity tolerance in vivo, but was the most sensitive at the cellular level (McCoy, 1987b). Nevertheless, the maize/Trisetum hybrid showed greater salinity tolerance, in vitro and in vivo, if compared with corn. Exposure of the N107BxT hybrid to 170 mM NaCl produced fresh weight increase reductions of 51% in vitro and 31% in vivo, which are lower than those observed for corn exposed to similar conditions.

Table 2. Water contents (%) of hybrid N107BxT plants, watered with tap water and with 170 mM NaCl

<table>
<thead>
<tr>
<th>Days after first watering</th>
<th>3</th>
<th>8</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>86.6 a</td>
<td>86.8 a</td>
<td>86.9 a</td>
<td>87.0 a</td>
</tr>
<tr>
<td>170 mM NaCl</td>
<td>85.5 a</td>
<td>86.9 a</td>
<td>85.4 b</td>
<td>84.3 b</td>
</tr>
</tbody>
</table>

Different letters in the column indicate significant differences between the means (Tuckey, α: 0.05).
i.e. 81.54% in vitro (Ramagopal, 1986) and 85% in vivo (Ashraf and McNeilly, 1989).

Given the tolerance found in the hybrid, it is an interesting material to investigate the mechanisms that confer this tolerance.

Acknowledgements

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References


GARCÍA M.D., MOLINA M. DEL C., 1997. Embriogénesis somática y regeneración de plantas a partir de embriones híbridos de Zea mays ssp. mays (2n = 20 y 40) y Tripsacum dactyloides. Libro Resúmenes del VI Congreso Nacional de Maíz, Pergamino, tomo I, pp. 79-86.


