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The use of electrolyte leakage in the evaluation of salinity tolerance at seedling stage in maize (*Zea mays* L.).

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Maize is classified as a salt-sensitive crop plant (Maas & Hoffman 1977). The response of maize to salinity varies depending on the stage of development (Kaddah & Ghowail 1964; Maas *et al.* 1983; Pasternak, Malach & Botovic 1985). Vegetative growth appears to be most sensitive to salinity, while plants are much less affected at later stages (Cramer 1994). The development of salt tolerance crop plant cultivars has been proposed as the most effective strategy to overcome this problem (Epstein & Rains, 1987). The resistance to abiotic stress in general and to salinity stress in particular is under polygenic control (Flowers & Yeo, 1995).

Munns (1993) has proposed a biphasic model of growth response to salinity. The growth reduction in the first phase is an effect of salt outside rather than inside the plant (osmotic phase). In the second phase, the concentration of toxic ions increases rapidly, especially in old leaves, which die as a result of a fast increase of the salt concentrations in the cell wall or cytoplasm when vacuoles can no longer sequester incoming salts (ionic phase). In this second phase, genotypes which vary in salt tolerance may respond differently as a result of their different abilities to exclude toxic ions or to sequester them in the vacuoles (Munns 1993).

The tolerance to salinity could be classified in three mechanisms:

1. Tolerance to osmotic stress: the mechanisms controlling this phase are not specific to salinity; they are associated with water stress.
2. Na^+ exclusion from leaf blades: the Na^+ is accumulating by the root and this protected the leaves to arise the salt to toxic level.
3. Tissue tolerance: the tissue tolerance to accumulated Na^+, the ion is compartmentalization at cellular and intracellular level to avoid toxic concentration within the cytoplasm (Munns & Tester, 2008).

Different typical agronomic selection parameters for salinity tolerance are being used: yield, survival, plant height (Noble and Rogers, 1992), leaf area (Franco et al., 1993), injury (Munns, 1993), relative growth rate in stress studies in different crops (He and Cramer, 1992). However, it is not yet possible to find any sensitive criterion that could reliably be used by breeders to improve salt tolerance of plants (Ashraf & Harris, 2004). Recently, several traits like: shoot K concentration (Bagci *et al*., 2007), photosynthetic capacity (Ashraf *et al*., 2007) and cell membrane stability (Aslam *et al*., 2006) in maize have been considered as a reliable parameter for salt tolerance studies.

Salt tolerance, studied by measuring cell membrane stability, has shown changes in the structure or composition of the membrane in genotypes with different response in salinity conditions. Salt sensitive cultivars show greater increase in the cell permeability

compared to salt tolerant cultivars. This trait could be reflected in the behaviour of the whole plant and could be a useful feature in a breeding program for developing salt tolerance genotypes (Mansour and Salama, 2004; Mansour *et. al*., 2005).

This paper examines the use of the electrolyte leakage (Cell membrane stability) trait in the selection at seedling stage that may be important in the screen for different mechanisms of tolerance in plants exposed to salinity.

Eight accessions/lines were used five of which were populations and three inbred lines. Seeds of the different genotypes were surface sterilized in 1% sodium hypochlorite solution for 5 minutes before experimentation, then rinsed with distilled water. Three seeds were planted in each pot containing perlit; these pots were put in trays with a nutrient solution. Two treatments were applied: control (cont.) where no ClNa solution was added and the other treatment receiving 100mM ClNa (salt). The experimental was carried out in controlled environmental room at 25 ºC, with 16 day length and with a relative humidity of 60%.

After 14 days of each salt treatment, the seedlings were harvested. The length for shoot and radicle (SL and RL, respectively) were recorded. Shoot and radicle were separated and the samples were dried for two days until constant weight, for dry mass determination (DS and DR respectively).

The cell membrane stability was estimated on the third leaf. A piece of leaf was cut, weighted and washed with distilled water to remove the solution from tissue, then the samples were immersed in 10ml of distilled water and placed for incubation at 10ºC for 24hs. After incubation samples were equilibrated to room temperature. Then, the electrical conductivity of the medium was recorded (EC1), with a portable EC meter (Consort C931). The samples were autoclaved for 15 min to kill all tissues, and after cooled to room temperature, the conductivity of the solutions was read again (EC2). Electrolyte leakage (%) was calculated as: EL = (EC1/ EC2) × 100. The electrolyte leakage was measured.

The data were subjected to an analysis of variance and the means were compared by the least significance differences test (LSD) at a 5% level (Sokal and Rolf, 1995).

The ANOVA pointed out that although the tested genotypes have shown significant and highly significant differences among themselves, in the salinity treatment, the comparison of both treatments has resulted non-significant for most of the traits that were tested, with exception of RL, EC1 and EL (Table 1).

Consequently, these traits would be extremely useful in salinity tolerance improvement programs, especially Root Length which has shown a major growth reduction compared to the controls. This apparently evidences the importance of the Root Length variable in the identification of a tolerant response, as pointed out by various authors (Rao and McNelly, 1999; Khan and McNelly, 2003).

Las mediciones de daño de membrana estarían asociadas con la susceptibilidad a la sal a nivel celular. El gráfico Nº1 muestra que el genotipo F564 podría ser considerado como susceptible, por que fue el que mayor valor de EC1 presentó y en consecuencia mayor daño por salinidad. En cambio, SC75 presentó el valor más bajo de EC1 por consiguiente, tendría una menor pérdida de electrolitos lo que indicaría un comportamiento tolerante. Sin embargo, cuando se analizan los parámetros de crecimiento, en especial el RL puede observarse que estos dos genotipos fueron los que menos pérdida de crecimiento sufrieron. En consecuencia, ambos genotipos sería tolerantes a salinidad pero asociada probablemente a mecanismos diferentes de tolerancia. La línea SC75 no habría sufrido gran daño en membrana debido probablemente a que no habría acumulado en forma

excesiva Sodio en la parte área, durante el tiempo de exposición a sal este ión se acumuló en raíz (mecanismo de exclusión de sodio). En cambio, F564 sufrió un importante daño en membrana, que podría asociarse a una acumulación de sodio en parte aérea (vacuola) sin afectar grandemente el metabolismo celular (tolerancia de tejidos al sodio). Measurement of membrane damage seems to be associated with salt sensitivity at cellular level. Figure 1 shows that genotype F564 could be considered sensitive because it showed the highest level of EC1 and, consequently, the most damage due to salinity. On the other hand, SC75 showed the lowest level of EC1 and therefore, it has a lower electrolyte leakage which indicates a tolerant behavior. However, when growth parameters are analyzed, in particular RL, it can be observed that these two genotypes were the ones that suffered the least growth loss. As a result, both genotypes appear to be tolerant to salinity but probably associated with different tolerance mechanisms. The SC75 line did not suffer great membrane damage, since it probably did not accumulate an excessive amount of Na^+ in the shoot during the period of exposure to salt, this ion was accumulated in the root (sodium exclusion mechanism). Instead, F564 suffered significant damage in the membrane, which could be associated with an accumulation of sodium in shoot (vacuole) without severely affecting the cellular metabolism (tissue tolerance to sodium).

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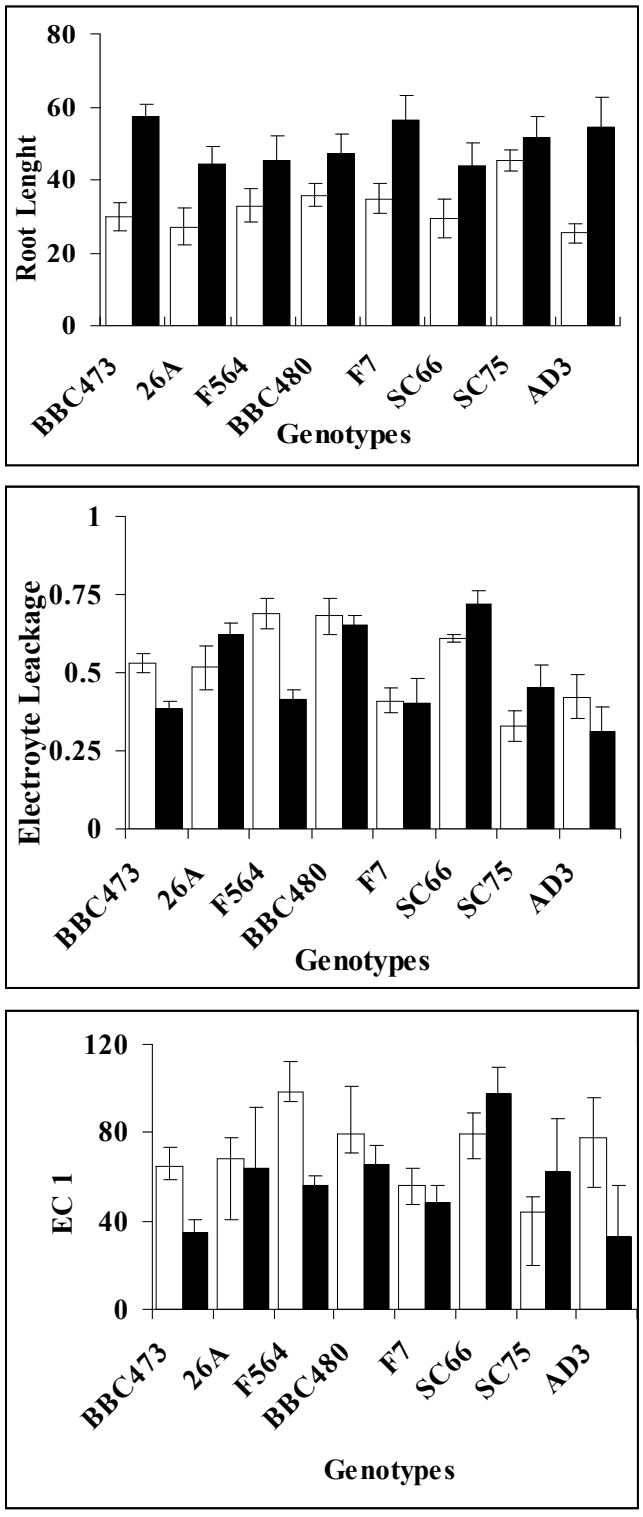

Figure 1: Average of each genotypes for control (black bars) and salt (white) treatments of the traits: Lenght of Root (RL), Electrolyte Leakage (EL) and Electrical Conductivity 1 (EC1). Vertical bars are the S.D. of four replications.