Research Paper

Salt stress affects germination, seedling growth and physiological responses differentially in eggplant cultivars (*Solanum melongena* L.)

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A B S T R A C T

Salinity tolerance levels and physiological changes were evaluated in four eggplant cultivars (*Solanum melongena* L.). Seeds and seedlings were exposed to increasing salinity levels. In vitro germination kinetics and young seedling growth divided the cultivars in a sensitive and a tolerant group. 'Adriatica' and 'Black Beauty' supported only moderate salt stress up to 40 mM NaCl while the tolerance level of 'Bonica' and 'Galine' was up to 80 mM NaCl. Effects of salinity levels (0, 20, 40, 80 and 160 mM of NaCl) were also tested in a greenhouse experiment. Increasing NaCl concentration increased strongly the levels of proline, malondialdehyde and soluble carbohydrates in the leaves of 'Adriatica' and 'Black Beauty'. In contrast, 'Bonica' and 'Galine' showed a decrease in soluble carbohydrates and a significant increase in starch under saline stress. The midday leaf water potential (ψ_l) and leaf osmotic potential (ψ_π) were significantly affected in sensitive cultivars and remained quite stable in tolerant cultivars under salt stress. Leaf Na^+ and Cl^- content was higher in sensitive than in tolerant cultivars. The leaf K^+, Ca^{2+} and Mg^{2+} contents were reduced under salt stress in sensitive cultivars. Under increasing salinity 'Bonica' and 'Galine' combine low leaf Na^+ accumulation with a high plant tolerance index (PTI) thus maintaining a normal level of growth, while 'Adriatica' and 'Black Beauty' accumulate significantly higher concentrations of leaf Na^+ and fail to maintain a normal level of growth from 80 mM NaCl on.

1. Introduction

Water scarcity and quality degradation are major constraints for agricultural development in the southern Mediterranean countries. Moreover, the introduction of irrigated agriculture in arid and semi-arid regions resulted in the development of secondary soil salinization. Facing salt stress, the plant uses different morphological and cellular responses to adapt. Thus, the search for traits related to salt tolerance is an important step for the selection of genotypes to improve their performance under these conditions.

The sensitivity of plants to salinity may depend on their developmental stage. Therefore, the study of salt tolerance during different growth phases is essential for detecting saline limits at each developmental stage (Zapata et al., 2004). Salinity reduced as well as delayed germination of solanaceous vegetables such as melon (Botia et al., 1998), tomato (Cuartero and Fernandez-Munoz, 1999) and eggplant (Akinci et al., 2004). Reduced seed germination induced by salt could be caused by dormancy induction, by osmotic stress or by specific ion toxicity (Shannon and Grieve, 1999).

Increasing salinity causes a significant decline in biomass production (Parida and Das, 2005) and thus decreases yields for a wide variety of crops all over the world. One of the earliest responses under salt stress is a leaf area reduction and the changed plant water status most likely leads to this initial growth reduction (Dash and Panda, 2001). Under salinity stress both osmotic and ionic effects affect the metabolism of plant cells in many ways. Specific effects of salt stress on plant metabolism have been related to the accumulation of toxic ions (Na^+ and Cl^-) or to K^+ and Ca^{2+} depletion (Munns et al., 2002). In addition, accumulation of Na^+ changes ion balances such as Na^+/Ca^{2+} and K^+/Na^+ ratios in plant cells. A high Na^+/Ca^{2+} ratio results in increased cell permeability. Ion disorder caused by salinity may also lead to changes in plant lipid metabolism (Kuiper, 1985). Lipid peroxidation, induced by free radicals, can increase the membrane fluidity and permeability and might finally lead to leaf necrosis (Halliwell, 1987).

Plants develop an array of mechanisms to cope with salinity. Under saline conditions, plants accumulate compatible solutes such as sugars, amino-acids, proteins and/or other compounds to protect themselves against the damage of the salinity and to accommodate the ionic balance in the vacuole in a process called osmotic adjustment (Yazici et al., 2007; Turkan and Demiral, 2009). Compartmentalization of toxic ions in different tissues is another possibility to enable metabolic functions and to tolerate higher amounts of salt in the soil (Munns and Tester,

2008)

Vegetables are generally considered as glycophytes and therefore susceptible to soil salinity (Colla et al., 2010; Shahbaz et al., 2012). Eggplant (*Solanum melongena* L.) is a vegetable crop of high importance in the Mediterranean region with Egypt and Turkey belonging to the top five of eggplant producing nations (FAO, 2015). A few studies have addressed the impact of salt stress on eggplant. Growth parameters and horticultural performance were considered to select more tolerant cultivars (Savvas and Lenz, 2000; Akinci et al., 2004). Hanachi et al. (2014) used chlorophyll *a* fluorescence to evaluate photosynthesis dysfunction under increasing salt stress in four eggplant cultivars. The salt stress induced an early reduction of Φ_{PSII} indicating a decrease in electron transport through Photosystem II in salt susceptible genotypes ('Adriatica' and 'Black Beauty') while Φ_{PSII} was not affected in tolerant cultivars ('Bonica' and 'Galine'). Yet, no information on how physiological and biochemical traits of eggplant cultivars with an opposing salt tolerance evolve under salt stress has been published.

The objective of this study was to evaluate the relative salt tolerance of four genotypes ('Adriatica', 'Black Beauty', 'Bonica' and 'Galine'). We investigated the salinity tolerance of these four eggplant cultivars by *in vitro* germination kinetics and seedling growth and by a greenhouse experiment. To interpret the cultivar reactions we analysed physiological parameters, osmotic adjustment and the accumulation of sodium and chloride in the leaves. We related growth performance to Na^+ accumulation in the leaves. This evaluation may facilitate screening for more tolerant genotypes.

2. Materials and methods

2.1. Plant material and experimental set-up

Four commercial eggplant (*S. melongena* L.) cultivars, two open-pollinated ('Adriatica' and 'Black Beauty') and two F1 hybrids ('Bonica' and 'Galine') were used as plant material. Two experiments were conducted, one in vitro germination and seedling growth experiment (Exp 1) and one greenhouse experiment (Exp 2).

2.1.1. Experiment 1

Seeds were surface-sterilized with 70% ethanol and rinsed with distilled water. Then, they were soaked in a solution of 0.02% Dreft (5–15% non-ionic surfactants, 15–30% anionic surfactants) and 5% HazTab (1,3,5 Dichloro-Triazine-Trionedihydrate-Dichlorosodium) for 20 min followed by a second soaking in a solution of mercuric chloride (0.5%) for 10 min. After three rinses with sterile distilled water the seeds were germinated on agar-solidified (0.8%) MS medium with 3% (w/v) sucrose in 0.7 L glass vessels. The pH was adjusted to 5.8 with 1 M NaOH before adding agar. Five concentrations of NaCl were added to the medium: 0 (control), 20, 40, 80 and 160 mM. Germination and seedling growth took place in a growth chamber at 28 °C under a 16 h photoperiod regime provided by cool-white fluorescent lamps with a photon fluency of 36 µmol m^{-2} s^{-1} at seedling level. For each treatment and cultivar four seeds per vessel were used, this in five replicates.

The germination (radicle emergence) and the time to germinate were determined at 24 h intervals for 7 days. Three parameters of germination were determined which included: final germination percentage; the mean germination time (MGT) and the mean daily germination (MDG). The mean germination time is calculated as follows: MGT = {(n$_1$t$_1$) + (n$_2$t$_2$) + ……. + (n$_x$t$_x$)}/X$_n$ where n$_1$ is the number of germinated seeds at the first day of germination, t$_1$ is the number of days from start to first germination, and X$_n$ is the total number of seeds germinated. The mean daily germination (MDG) is the final germination percentage per number of days to final germination.

After 6 weeks one seedling per vessel was randomly sampled and seedling length, number of leaves, aerial fresh (FW) and dry weight (DW) were measured. The aerial part of the plant (shoots and leaves) was weighed (FW) and then dried in a forced-draft oven at 80 °C for

24 h and re-weighed (DW). Tissue water content (TWC) was calculated as the (FW-DW/FW) ratio. Minimal two seedlings per vessel were pooled, ground in liquid nitrogen and stored at −80 °C until metabolite analysis.

2.1.2. Experiment 2

Seeds were sown into 80 mL plug trays containing a peat-based medium in a growth chamber at a constant temperature of 25 °C, RH of 70%, photon flux density of 150 µmol m^{-2} s^{-1} and photoperiod of 16 h. Seedlings were selected for uniformity and transplanted into 2 L plastic pots at the appearance of the second true leaf. Plants were transferred to a heated glasshouse with a minimum temperature set-point of 21 °C (located at 51°02′N, 03°42′E) and were fertigated with 250 mL full-strength Hoagland's solution (Hoagland and Arnon, 1950), twice a week. The temperature ranged between 22 °C and 27 °C while the daily maximum photon flux density averaged 340 µmol m^{-2} s^{-1} (quantum sensor SKP215, Skye at plant canopy and connected to a data logger, type DL3000, Delta-T, UK; data were logged every 10 min). Shading screens were used to prevent direct sunlight to the plants. Five salinity treatments were applied starting 36 days (4th leaf stage) after the transfer to the greenhouse. NaCl was added at 0 (control), 20, 40, 80 and 160 mM to a full-strength Hoagland's solution with electrical conductivity of 1.18, 1.42, 2.75, 3.05, and 5.21 dS m^{-1} respectively. Irrigation frequency and dose per plant remained unchanged.

Each treatment was applied to 20 plants per cultivar. The experiment was designed as a randomized complete block design, with four blocks. Each experimental unit contained five plants. After 30 days of saline stress eight plants (2 plants per block) were taken at random for each treatment. The height and the number of leaves were measured. Dry weight (DW) was determined after 48 h drying at 60 °C in a forced-draft oven. The tissue water content of the aerial biomass (TWC) was calculated as (FW-DW/FW). Then, leaves were milled and stored at 4 °C until leaf mineral analysis. For metabolite analysis, fully developed upper leaves (2 leaves/replicate in a bulked sample) were harvested between 12 h and 14 h. Leaf material was ground in liquid nitrogen and stored at −80 °C until analysis.

A plant tolerance index (PTI) was calculated based on the total fresh weight (FW) in salt stressed plants per total FW in control plants.

2.2. Plant water status

The midday leaf water potential (Ψ_l) (11 h–12 h) of the youngest fully-expanded leaves was determined with a Scholander pressure chamber (model 1000, PMS Instrument Company, Albany, OR, USA). The leaf osmotic potential (Ψ_π) was determined according to the method of Callister et al. (2006) using an osmometer (Fiske One-Ten, Fiske Associates, Howard, USA). The analysis was done in four replicates per treatment.

2.3. Metabolite analysis

Proline was determined according to Bates et al. (1973). Plant tissue (500 mg) was extracted with 10 mL of 3% (w/v) sulfosalicylic acid. After filtration, 2 mL acid ninhydrin and 2 mL glacial acetic acid were added to the extracts (2 mL) and this mixture was kept at 100 °C for 1 h in a water bath, then the reaction was stopped in an ice-bath. The formed chromophore was extracted from the acid aqueous solution by cold toluene (4 mL) and the absorbance was measured at λ = 520 nm (InfiniteM200 TECAN Group Ltd., Switzerland). The proline concentration was determined using a calibration curve and expressed as µg proline g^{-1} FW.

Lipid peroxidation was measured as the amount of malondialdehyde (MDA) determined by the thiobarbituric acid (TBA) reaction (Hodges et al., 1999). Leaf material (1 g) was homogenized in 25 mL 80% ethanol, followed by centrifugation at 3000*g* for 10 min. A 1 mL aliquot of sample extract was added to 1 mL of thiobarbituric acid (TBA, 0.65%