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Research article

Bio-priming mitigates detrimental effects of salinity on maize improving antioxidant defense and preserving photosynthetic efficiency

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ABSTRACT

Salinity is an abiotic stress which seriously affects crop production over the world, particularly in arid and semi-arid regions, with harmful effects on germination, growth and yield. Maize (*Zea mays* L.), cultivated in a wide spectrum of soil and climatic conditions, is the third most important cereal crop after rice and wheat, moderately sensitive to salt stress. A saline level more than 250 mM NaCl damages maize plants, causing severe wilting. In this study, the effects of hydro-priming (distilled water) and bio-priming (*Rosmarinus officinalis* L. and *Artemisia* L. leaf extracts) on seed germination and seedling growth of maize, under 100 mM NaCl salinity were investigated. The factorial experiments were carried out in greenhouse under controlled condition (25 °C in 12/12 h day/night) based on a completely randomized design with three replicates. Results showed that both hydro- and bio-priming increased germination percentage and germination indexes in maize seeds. Rosmarinus extract was the most effective in inducing salt resistance in 30 days old seedlings, with beneficial effects in the strengthening of the antioxidant system and in the maintenance of a higher photosynthetic efficiency under salt stress condition.

1. Introduction

Salinity is one of the major abiotic stresses that negatively affect crop productivity. More than 800 million hectares of land worldwide are affected by either salinity (397 million hectares) or sodicity (434 million hectares) with a decline, by more than 50 percent, in average yield of the major crop plants (FAO, 2011; Munns and Tester, 2008). Salt stress occurs in areas where soils are naturally high in salts and precipitations are low and/or where irrigation, hydraulic lifting of salty underground water, or invasion of sea water in coastal areas bring salt to the surface soil. NaCl is the predominant salt causing salinization worldwide (Munns and Tester, 2008). The most widely accepted effects of salinity are decrease in germination percentage, germination rate and in growth and metabolism of seedlings by creating an external osmotic potential that prevents water uptake, or by causing specific ion toxicity and ion imbalance. Salinity also affects cellular metabolism including photosynthesis and synthesis of compatible solutes called “osmolytes” like proline, sugars (Amirjani, 2011) and proteins (Sen and Alikamanoglu, 2011).

Maize (*Zea mays* L.) is the third most important cereal crop after rice and wheat and it is grown under a wide spectrum of soil and climatic conditions. It is an important C4 plant from the Poaceae family, moderately sensitive to salt stress (Farooq et al., 2015). A saline level containing more than 250 mM NaCl may damage maize plants and stunt growth causing severe wilting (Menezes-Benavente et al., 2004). For this reason, the aim of this work was to find a method that could alert and/or attenuate the negative impact of salts increasing the tolerance to salinity in maize plants. Seed priming is an easy, low cost and low risk technique recently used to overcome the salinity problem in agricultural lands (Ibrahim, 2016; Chen and Arora, 2013). It is a pre-sowing treatment and the most important priming treatments include halo-priming (soaking seeds in inorganic salt solutions), solid matrix priming (treatment of seeds with solid matrices), osmo-priming (soaking seeds in solutions of different organic osmotic) and bio-priming (using a priming mixture integrated with bioactive molecules or beneficial microorganisms) (Nouman et al., 2014). All the above listed priming are able to 1) stimulate metabolic processes involved in the early phases of germination, producing high germination rate and great germination percentage 2) induce uniformity and faster emergence of seedlings from primed seeds, bring vigorous growth in adverse conditions (Imran et al., 2013).

Maher et al. (2013) and Tzortzakis (2009) indicated also that seed priming in fenugreek, endive and chicory increased final germination percentage, germination speed and radicle length over the non-primed

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treatments in saline conditions. Generally, farmers facing with saline problems, cannot reclaim soil, or use expensive plant hormones, antioxidants or nutrients for seed priming (Basra et al., 2011; Imran et al., 2013). So, there is the need to explore new plant growth enhancers, naturals and environmentally friendly which should be reliable and economically sustainable under prevailing salinity conditions. This study was planned to investigate the potential of *Rosmarinus officinalis* L. and *Artemisia* L. aqueous extracts as seed bio-priming agents. The genotypes tested in this study have a high intraspecific populations genetic similarity (le Floc'h, 1983; Boussaïd et al., 2006). These two species, mostly found in arid and semi-arid areas, are widely distributed in Mediterranean countries, and represent new sources of natural antioxidant and antimicrobial agents. Thus, we hypothesized that seed priming with these natural extracts may alleviate salt stress in germinating maize seeds and improve seedling establishment by modulating antioxidants, photosynthetic pigments, ionic homeostasis and photosynthesis. Both extracts can be a critical tool for enhancing productivity in organic/sustainable agriculture improving the circular economy in Tunisia.

2. Materials and methods

2.1. Extract preparation and chemical characterization

Rosmarinus (*Rosmarinus officinalis* L.) and Artemisia (*Artemisia erba alba* L.) plants growing wild in Tunisia were collected between February and March 2016 from the region of Chouachi- HadjebAyoun (Tunisia 35° 23′ North 9° 32′ Est) and identified according to the flora of Tunisia (Pottier-Alapetite, 1981). Fresh leaves were dried and grounded and the aqueous extracts were prepared by soaking the dried leaves, overnight in distilled water (1:10 w/v) (Durak et al., 2016). The suspensions were filtered with Whatman's paper. Extracts have been analyzed by HPLC analysis with a Knauer (Berlin, Germany) apparatus interfaced to a DAD detector (model 2600). HPLC-DAD technique is commonly used to detect antioxidants in many matrix (Giuffrè, 2013). For separation a binary gradient was prepared: (A) bi-deionized water and (B) acetonitrile, both were acidified at pH 3 with formic acid. The applied gradient was: 0–20 min, 95% A and 5% B; 20–50 min, the eluent B increased from 5 to 40%; 50–60 min, eluent B increased from 40% to 95%; 60–65 min, the eluent B decreased from 95% to 5%; 65–70 min 95% A and 5% B in isocratic. The analysis was performed with a constant flow rate of 1 ml/min. A Knauer C18 Eurosphere II separation column (Berlin, Germany) was used (250 mm length x 4.6 mm internal diameter x 5 µm particle size). All standard components (purity ≥ 97%) were purchased from Sigma Co. (St. Louis, MO). All solvents and reagents (HPLC grade) were purchased from Panreac (Barcelona, Spain). The identification of unknown components in the extracts were performed by comparison the retention times of the detected compounds with those of appropriate standards.

2.2. Seed priming

For each treatment, 30 healthy seeds were surface sterilized with 5% sodium hypochlorite for 5 min and then rinsed with sterile bi-distilled water. Aqueous extracts of Rosmarinus (RP) and Artemisia (AP) were used for seed priming. Maize seeds were soaked in the extracts, ratio 1:5 (w/v) for 24 h in darkness at room temperature (ur Rehman et al., 2015). The Hydro-primed (HP), bio-primed (RP, AP) and the un-primed (CNP) seeds were dried back to their original moisture contents at room temperature and were used as control. The experiments were performed in triplicate.

2.3. Assessment of seed germination and morphological, physiological and biochemical responses of seedlings

After priming, seed germination tests were carried out. 10 seeds for each treatment (priming and control) were placed in plastic pots (26 cm diameter × 27 cm height) filled with sand and equilibrated with water (control) or NaCl 100 mM. 70% of field capacity was maintained with distilled water. Each treatment was replicated three times. Experiments were carried out in climatic chamber at 25 °C in a 12/12-h photoperiod for 30 d. Seeds were considered germinated when a visible coleoptile protrusion was observed. The germinated seeds were counted daily and the germination percentage was calculated at the 7 days. Germination index (GI), mean germination time (MGT), time to reach 50% germination (T_{50}) and total germination percentage TG were calculated as follow:

$$GI = \Sigma(G_t/T_t);\ MGT = \Sigma(G_t \times T_t)/\Sigma G_t \text{ and } T50 = t_i + [(N/2\text{-}n_i)\ (t_j\ \text{-}t_i)]/(n_j\text{-}n_i).$$

G_t is the number of germinated seeds on day t, T_t is the time corresponding to G_t in days, N is the final number of germination. ni and nj are the cumulative number of seeds germinated by counts adjacent at times when ni < N/2 < nj (Zhang et al., 2007).

Root length and shoot height were measured manually with a ruler and dry weights were determined after drying at 80 °C for 24 h.

Table 1
Chemical composition of leaf aqueous extracts of *Rosmarinus officinalis* L. and *Artemisia* L. Values are the means of three replicates ± Standard Deviation (SD).

Rosmarinus extract	mg/l	SD
Gallic Acid	3.864	0.018
Neoclorogenic Ac.	4.092	0.097
Clorogenic Acid	1.961	0.062
Siringic Acid	1.969	0.061
Rutin	32.623	0.060
Rosmarinic Acid	99.116	0.004
Apigenin	4.089	0.050
Carnosol	1.047	0.052
Carnosic Acid	9.183	0.052

Artemisia extract	mg/l	SD
Gallic Acid	1.655	0.005
Protocatechic Acid	3.093	0.028
Clorogenic Acid	32.395	0.134
Siringic Acid	6.506	0.108
Narirutin	5.043	0.060
Naringin	7.838	0.035
3,4-Di-O-caffeoylquinic Acid	1.907	0.013
Luteolin	0.518	0.003
Apigenin	2.175	0.010
Kaempferol	0.384	0.019

2.4. Photosynthetic pigments in seedlings

Fresh leaves (0.05 g) were extracted with 2.5 ml of pure ethanol and incubated for 24 h at 4 °C in the dark. After, the samples were centrifuged for 10 min at 7000 rpm. For chlorophyll and carotenoid analysis, the absorbance of supernatants was recorded at 649 nm, 665 nm, and 470 nm and their concentrations (mg/g fresh weight) were calculated using Lichtenthaler's equations. (Lichtenthaler, 1987). Anthocyanins were extracted incubating 20 mg of fresh leaf in 0.5 ml of methanol: HCl (99:1). After 24 h incubation at 4 °C, samples were centrifuged at 6000 g for 10 min at 4 °C (Panuccio et al., 2016). The absorbance was read at 530 and 657 nm and anthocyanin content (µg anthocyanin/g fresh weight) was calculated according to the following equation: [A530 nm – (0,025*A657 nm)*ml of extract]/g fresh weight.

2.5. Chlorophyll fluorescence imaging

Photosynthetic efficiency of primed and un-primed seedlings in absence and in presence of salinity was evaluated by using an Imaging