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Physiological and antioxidant responses of the perennial halophyte *Crithmum maritimum* to salinity

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Abstract

Physiological behavior and antioxidant responses to salinity were studied in *Crithmum maritimum*, a local halophyte naturally growing on rocky coasts. The plant growth was significantly improved at moderate salt levels (50 mM NaCl), but was drastically reduced at 200 mM NaCl. The stimulation of biomass production at 50 mM NaCl was associated with enhanced root length and leaf number. Tissue hydration seemed unaffected by salinity, despite Na^+ and Cl^- were largely accumulated in shoots. The highest salinity (200 mM NaCl) induced mineral nutrition disturbance within the plant shoots, as their Ca^{2+}, Mg^{2+}, and K^+ concentrations significantly declined. However, *C. maritimum* displayed high uptake selectivity for the latter. Monitoring lipid peroxidation showed that both root and shoot malonyldialdehyde (MDA) contents of plants cultivated at the optimal salt concentration (50 mM NaCl) were lower than control ones. This was related to enhanced activities of antioxidant enzymes, like superoxide dismutase (SOD) (EC 1.15.1.1), catalase (EC 1.111.1.6), and peroxidase (EC 1.111.1.7), especially in shoots. The limitation of the plant growth at 200 mM NaCl was concomitant with lesser efficiency of these protective enzymes, but MDA levels in both roots and shoots remained close to control ones.

Keywords: Antioxidant enzymes; Halophyte; K^+ over Na^+ selectivity; Lipid peroxidation; Salt tolerance

1. Introduction

Though soil/water salinity is an old phenomenon, this environmental problem has been aggravated by modern intensive agricultural practices, and the increasing irrigation needs. Today, 20% of the world cultivated areas and nearly half of all irrigated lands are affected by salinity [1]. At this trend, it is estimated that the production of the agricultural lands will not provide humans with sufficient foods from 2020 to 2030 [2].

Salt accumulation is the primary factor depressing the yields of cultivated plants, which are almost non halophytic [3–5]. It is assumed that salt stress cause an imbalance of the cellular ions resulting in ion toxicity and osmotic stress [6], thus affecting the plant growth, morphology, and survival [7]. Mechanisms of salt tolerance are of two main types: those minimising the entry of salt into the plant, and those minimising the concentration of salt in the cytoplasm. Halophytes, naturally salt tolerant plants, have both types of mechanisms. They exclude salt well, but effectively compartmentalise in vacuoles the salt that inevitably gets in. This allows them to growth of long period of time in saline soil.

The elucidation of physiological and biochemical mechanisms are critical, before trying to introduce genetic and environmental improvements to this stress [8]. Salt-tolerance mechanisms are quite complex, including osmotic adjustment, compartmentation of toxic ions [9–13], metabolite accumulation, ion homeostasis, redox control, and scavenging of activated oxygen species (AOS) [14,15].

Salinity generates oxidative stress in plant tissues [16–18], though the origin of oxidative damage is still confusing

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[19]. It is likely that salt stress limits gas exchange and thereby CO_2 supply to the leaf [20,21]. One consequence is the over-reduction of the photosynthetic electron transport chain [22]. This induces the generation of activated oxygen species, such as singlet oxygen, superoxide anion, hydrogen peroxide, and hydroxyl radical [23,24]. Levels of AOS are regulated by their rates of generation, their rate of reaction with target substances, such as proteins, lipids, and/or nucleic acids, their potential rate of degradation, and their rate of scavenging/neutralizing by enzymatic and/or non enzymatic antioxidants [25].

Several enzymes are involved in the detoxification of AOS. Superoxide dismutase (SOD) (EC 1.15.1.1) converts superoxide to H_2O_2. Hydrogen peroxide is scavenged by catalase, CAT (EC 1.111.1.6) and different classes of peroxidases, POD (EC 1.111.1.7) [26]. The capacity to scavenge AOS and to reduce their damaging effects on macromolecules appears to represent an important stress-tolerance trait [27]. A close correlation between the antioxidant capacity and NaCl tolerance has been demonstrated in numerous crops, such as pea [28], cotton [24], rice [29] and foxtail millet [30]. In addition, recent reports on the responses of plant antioxidant enzyme to salinity showed varying activity patterns according to the species and analysed tissues [31].

Crithmum maritimum, or sea fennel, (Apiaceae) is a fleshy aromatic, perennial littoral halophyte, naturally thriving on rocky coasts. Besides its obvious interest as a naturally salt-tolerant plant, this species shows considerable economical and medicinal potentials. Indeed, *C. maritimum* leaves which are rich in several compounds, such as vitamin C, carotenoids, flavonoids, as well as bioactive substances which could be used for aromatic, medicinal, antimicrobial, and insecticide purposes [32–34]. Its seeds contain also appreciable amounts of oil, potentially edible due to its fatty acid composition, close to olive-oil [35].

Preliminary studies have shown that *C. maritimum* is a facultative halophyte: its growth was significantly reduced when plants were grown on sandy soil irrigated with nutrient solution supplied with NaCl, ranging from 100 to 300 mM NaCl [36]. The aim of this study was to investigate the changes in growth parameters, the ionic and water status and the activities of several antioxidant enzymes of this halophyte, when subjected hydroponically to NaCl stress.

2. Materials and methods

2.1. Plant material and culture conditions

In its natural biotope (rocky coasts), *C. maritimum* is characterised by an important seed yield with a homogenous seed ripening and a suitable size. Woody at the base, the plant height reaches up to up to 50 cm. The leaves are deltate, biternately or triternately compound, with 1–4 cm width segments. The flowers are in compound umbels, while the ovoid-oblong fruit is yellowish or purplish and approximately 6 mm long [37].

Seeds were collected in December 2002 from rocky coasts of Tabarka (160 km north of Tunisia). Seeds were sterilized in 0.2% (w/v) sodium hypochlorite for 3 min and germinated on filter paper in Petri dishes in a growth chamber at controlled conditions (15–25 °C Temperature, 70–90% relative humidity, 16–8 h-night–day photoperiod, and 200 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic active radiation, PAR). Two-leaf seedlings (58 individuals) were transferred to 5 l-plastic pots (5 plants per pot) and were hydroponically cultivated, using aerated Hewitt nutrient solution (pH 7.3, EC 2.7 $mS\ cm^{-1}$) [38], containing macronutrients (mM): $MgSO_4$ (1.5), KH_2PO_4 (1.6), K_2HPO_4 (0.4), KNO_3 (3), NH_4NO_3 (2), $Ca(NO_3)_2$ (3.5). The medium contained also iron as complex EDTA–K–Fe [39] and micronutrients as a mixture of salts: $MnCl_2$, $CuSO_45H_2O$, $ZnSO_4{,}7H_2O$, $Mo_7O_{24}(NH_4)_6$, $4H_2O$ and H_3BO_3 [40]. An initial harvest was made on 35-day-old plants (10 plants). The remaining plants were separated in three groups irrigated with a nutrient solution supplemented with different concentrations of NaCl (0, 50 and 200 mM). To avoid osmotic shock, salt concentrations daily stepwise increased with 50 mM NaCl. The nutrient solutions were replaced each 4 days. Sixteen plants per salt-treatment were used: 10 for measuring the physiological parameters, 3 for the antioxidative enzyme assays, and 3 for the determination of malonyldialdehyde (MDA) contents.

The final harvest occurred after 2 months of salt-treatment. For the determination of the dry weight (DW), plants were then separated in shoots and roots, and oven-dried at 60 °C (for 3 days). Besides, fresh shoot and root samples from each plant were immediately frozen in liquid nitrogen and stored at −80 °C, until performing the biochemical analysis.

2.2. Ions concentrations

Ion extraction was achieved in 0.5% HNO_3. Chloride was assayed by coulometry (Buchler chloridometer), Na and K by flame emission photometry (Corning, UK), and Ca and Mg by atomic absorption spectrophotometry (Instrumental laboratory, USA).

2.3. Plant water relations

Shoot or root water content was calculated as (FW - DW)/FW, where FW and DW represent the fresh and dry weight, respectively. Leaf water potential (Ψw) was measured (first fully turgescent leaf) using a pressure chamber (PMS Instrument Co., Corvallis, Oregon, USA), according to Sholander et al. [41]. Relative water content (RWC) was estimated by recording the turgid weight of fresh shoot or root samples by keeping them in water for 4 h, followed by their drying in hot air oven till constant