Plant Physiology and Biochemistry 118 (2017) 609–617

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Potassium deficiency alters growth, photosynthetic performance, secondary metabolites content, and related antioxidant capacity in *Sulla carnosa* grown under moderate salinity

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ARTICLE INFO

Article history:
Received 28 April 2017
Received in revised form
19 July 2017
Accepted 2 August 2017
Available online 4 August 2017

Keywords:
Antioxidants
Photosynthesis
Potassium deficiency
Salinity
Sulla carnosa

ABSTRACT

Salinity and K^+ deficiency are two environmental constraints that generally occur simultaneously under field conditions, resulting in severe limitation of plant growth and productivity. The present study aimed at investigating the effects of salinity, either separately applied or in combination with K^+ deficiency, on growth, photosynthetic performance, secondary metabolites content, and related antioxidant capacity in *Sulla carnosa*. Seedlings were grown hydroponically under sufficient (6000 μM) or low (60 μM) K^+ supply with 100 mM NaCl (C + S and D + S treatments, respectively). Either alone or combined with K^+ deficiency, salinity significantly restricted the plant growth. K^+ deficiency further increased salt impact on the photosynthetic activity of *S. carnosa*, but this species displayed mechanisms that play a role in protecting photosynthetic machinery (including non photochemical quenching and antioxidant activity). In contrast to plants subjected to salt stress alone, higher accumulation of phenolic compounds was likely related to antioxidative defence mechanism in plants grown under combined effects of two stresses. As a whole, these data suggest that K^+ deficiency increases the deleterious effects of salt stress. The quantitative and qualitative alteration of phenolic composition and the enhancement of related antioxidant capacity may be of crucial significance for *S. carnosa* plants growing under salinity and K^+ deficient conditions.

1. Introduction

Salt stress is one of the biggest environmental factors impacting plant growth and productivity in many parts of the world, particularly in irrigated lands of arid and semi-arid regions (Hafsi et al., 2007; Mostek et al., 2015). This constraint affects more than 800 million hectares of land, representing more than 6% of the total global area of the Earth (Munns and Tester, 2008). In Tunisia, about 1.5 million ha are salt affected which represent 10% of the whole territory (Hachicha et al., 1994).

The competitive restriction of K^+ uptake by Na^+ into plant cells, due to physicochemical similarities between these two cations, is among the major effects caused by salinity (Rubio et al., 1995; Hafsi et al., 2007). In fact, NaCl excess in the medium can cause a down-regulation of genes involved in K^+ transport (Zhu, 2003). In addition, Shabala et al. (2003) demonstrated that the depolarization of the plasma membrane with a consequent higher K^+ efflux may also occur because of NaCl-exposure. Lipid peroxidation and K^+ loss from cells by activating K^+ efflux channels is another consequence of over-production of reactive oxygen species (ROS) induced by salinity (Demidchik et al., 2003; Cuin and Shabala, 2007). Therefore, to survive under salinity conditions, it is pivotal for plants to maintain K^+ homeostasis. In this context, a strong relationship between K^+ status in roots and salinity tolerance has been found in barley (Chen et al., 2005, 2007a, 2007b) and wheat (Cuin et al., 2012). Hafsi et al. (2007) reported that the tolerance of *Hordeum maritimum* to salinity stress may be related to the improvement of K^+ transport towards shoots and an increase of its use efficiency for biomass production. More recently, a correlation between salinity tolerance and the capacity of photosynthetically active mesophyll cells to retain K^+ was found in a screening experiment conducted on wheat and barley genotypes (Wu et al., 2013, 2014a, 2014b).

At the cellular level, K^+ plays important roles including

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http://dx.doi.org/10.1016/j.plaphy.2017.08.002
0981-9428/

activation of enzymes, stabilization of protein synthesis, neutralization of negatively charged proteins, and maintenance of cytosolic pH homeostasis (Shabala, 2003; Dreyer and Uozum, 2011). Therefore, several authors suggest that the regulation of Na^+ over K^+ selectivity is pivotal for maintaining an adequate K^+/Na^+ ratio in the cytoplasm, and hence preserving biochemical and biophysical-K^+ dependent processes (Munns and Tester, 2008; Shabala and Cuin, 2008). It was shown that selection of plants with higher K^+/Na^+ ratios in their tissues may be sufficient to select salt-tolerant genotypes (Chen et al., 2007a, b). Evidence has shown that the addition of K^+ can mitigate the deleterious effects of salt stress abiotic (Cakmak, 2005; Degl'Innocenti et al., 2009; Abbasi et al., 2015).

It was observed in several studies that photosynthesis, one of the most important metabolic processes in plants, was sensitive to salt stress (Koyro, 2006; Degl'Innocenti et al., 2009; Huang et al., 2014). A reduction in net CO_2 assimilation rate, transpiration rate, and stomatal conductance was frequently observed (Ouerghi et al., 2000; Liao and Guizhu, 2007; Degl'Innocenti et al., 2009). Salt-induced effects on photosynthesis were mostly due to a low osmotic potential of the soil solution, specific ion toxicities, nutritional imbalances or a combination of these factors (Ashraf, 1994; Zhu, 2003).

Salinity leads to increased ROS production in plant cells such as singlet oxygen, hydrogen peroxide molecules, superoxide, and hydroxyl radicals. These reactive oxygen species are produced in different sites including chloroplasts, mitochondria, peroxisomes, the endoplasmic reticulum, and plasma membranes (del Río et al., 2006; Foyer and Noctor, 2009). ROS overproduction can lead to oxidative damage to lipids, proteins, nucleic acids, and photosynthetic components (Meloni et al., 2003; Implay, 2003) or even to cell death (Jones, 2000). In order to reduce the deleterious effects of ROS, plant cells contain different protective mechanisms either through anti-oxidative enzymes or anti-oxidant secondary metabolites (Sharma and Dubey, 2005). Numerous non-enzymatic antioxidants such as α-tocopherol, β-carotene, ascorbate, and glutathione (Kim et al., 2001), and polyphenols (Ksouri et al., 2007; Valifard et al., 2014) are implicated in ROS-scavenging. Polyphenol synthesis and accumulation are stimulated in plants under salt stress, which might play an important role in ROS scavenging (Taârit et al., 2012). These are attributable to (i) the high reactivity of polyphenols as hydrogen or electron donors (ii) the capacity of these compounds in chelating transition metal ions, and (iii) the ability of radicals derived from polyphenols to stabilize and delocalize the unpaired electron (Huang et al., 2005).

Literature related to the interaction between salt stress and K^+ availability is still limited. In a previous work (Hafsi et al., 2010) we showed that adding 100 mM NaCl salinity was beneficial for the facultative halophyte *H. maritimum* when exposed to K^+ deficiency, due to the enhancement of the plant antioxidant response. More recently, we demonstrated in *Sulla carnosa* that K^+ deficiency modulates the composition of secondary metabolites and their antioxidant properties (Hafsi et al., 2016). Nevertheless, data related to polyphenol accumulation and related antioxidative activities of plants subjected to salt-K^+ deficiency mixed stresses are still scarce. In this study, we investigated the effects of salinity in combination with low or sufficient K^+ supply on growth, photosynthesis, polyphenolic compound contents, and related antioxidative capacities in the legume *S. carnosa*. *Sulla* constitutes an important genetic resource and contributes to pastoral production particularly in semi-arid regions because of its drought tolerance and enrichment of soil due to its nitrogen fixing capacity (Trifi-Farah et al., 2002). Furthermore, the genus *Sulla* contains various chemical constituents. To date, 155 compounds have been isolated through different chromatography methods, including flavonoids, triterpenes, coumarins, lignanoids, nitrogen compounds, sterols, carbohydrates, fatty compounds, and benzofuran which contribute to the antioxidant, anti-tumor, anti-aging, anti-diabetic, and anti-hypertensive properties of these plants (Dong et al., 2013).

2. Material and methods

2.1. Plant material and growth conditions

Seeds of *S. carnosa* were collected from Kalbia sabkha (a saline area in the center of Tunisia bioclimatic climate: semiarid, altitude: 34 m, location: 10°8′29″ N, 35°48′26″ E). Seeds were scarified, disinfected for 2 min with 1% NaClO and washed several times with distilled water. After, they were germinated in Petri dishes on filter paper moistened with distilled water. Three-day-old seedlings were transferred into plastic pots (14 plants pot^{-1}) and irrigated with 5 L of modified Hewitt's nutrient solution (Hewitt, 1966). The composition of nutrient solution was: 1.5 mM $MgSO_4$, $7H_2O$, 3.5 mM Ca $(NO_3)_2$, $4H_2O$, 5.4 mM $NaNO_3$, 2 mM $NH_4H_2PO_4$, and 6 mM KCl for macronutrients. The micronutrients (μM) were: Mn (0.5), Cu (0.04), Zn (0.05), B (0.5), Mo (0.02) (Arnon and Hoagland, 1940) and Fe (3) as Na_2-Fe-EDTA complex.

After 28 days pretreatment period, plants were divided into three lots (three replicates for each lot). The following treatments: C = control (complete medium (CM) containing 6 mM K^+), C + S = salt treatment (CM containing 6 mM K^+ with 100 mM NaCl), and D + S = interactive treatment (CM containing 60 μM K^+ and 100 mM NaCl) were used. The culture was performed in a greenhouse with day/night temperatures of 25°C/18 °C, a 16 h photoperiod, a photon flux density of 400 μmol m^{-2} s^{-1}, and a relative humidity of 70–75%. At the end of treatment period (30 days), plants were harvested and divided into roots, stems, and leaves. The fresh weights (FW) were immediately determined. Samples were then oven-dried for 48 h at 60 °C and dry weight (DW) was measured.

2.2. Pigment determination

After extraction in 80% acetone, chlorophyll and carotenoid concentrations (mg g^{-1} FW) were quantified spectrophotometrically from fresh leaves following the method of Arnon (1949).

2.3. Gas exchange and chlorophyll fluorescence measurements

Photosynthetic gas exchange was determined with a portable photosynthesis system (LCA4) (Bio-Scientific, Great Amwell, Herts, UK) at the end of the treatment period. Measurements were carried out between 10:00 and 13:00 h on the youngest fully emerged leaves at photosynthetically active radiation 1350 μmol m^{-2} s^{-1} ± 249, 27 ± 2 °C leaf temperature, 65 ± 5% relative humidity, and 380 μmol mol^{-1} ambient CO_2 concentration. Parameters of chlorophyll fluorescence were measured using a modulated chlorophyll fluorimeter (OS1-FL) following the method described by Genty et al. (1989). The minimal (F_0) and maximal (F_m) Chl a fluorescence were assessed in leaves after 20 min of dark adaptation. The following ratios were calculated according to Maxwell and Johnson (2000). The maximum quantum yield of PSII was calculated as $F_v/F_m = (F_m - F_0)/F_m$. The relative quantum yield of PSII at steady-state was determined as $\Phi_{PSII} = (F'_m - F_s)/F'_m$, where F_s and F'm are fluorescence at steady-state and maximum fluorescence in the light, respectively. Non-photochemical quenching of fluorescence (NPQ) was calculated as $NPQ = (F_m - F'_m)/F'_m$.