

Chapter 2

UTILIZATION OF SEAWEED IN SOIL FERTILIZATION-SALT TOLERANCE

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ABSTRACT

Salinity affects crop production worldwide. *Ascophyllum nodosum*, brown seaweed, has been used for centuries as a bio-fertilizer and/or bio-stimulant to promote plant growth and improve plant tolerance to biotic and abiotic stresses. However, the mechanisms of its bio-stimulatory activity are not well understood.

In this experiment, we investigated the effect of *A. nodosum* in alleviating the effects of salinity on tomato (*Solanum lycopersicum*) plants grown at 0-200 mM NaCl.

Results showed that *A. nodosum* promoted tomato plant growth under saline conditions due to the maintenance of the Na⁺/K⁺ balance. Its extract also interfered with Zn⁺ leaf concentration.

Keywords: tomato, salinity, seaweed, stress tolerance, *Ascophyllum nodosum*

INTRODUCTION

For centuries, whole seaweeds and processed or purified concentrates of seaweeds have been used in agriculture to improve stress tolerance (Crouch et al. 1990) in plants and animals. *Ascophyllum nodosum*, *Laminaria*, *Fucus* and *Ecklonia*, are commonly used. Their growth-promoting effects are mediated by naturally occurring bio-stimulatory components, including essential micronutrients, traces of vitamins, and complex organic molecules, which may act in similar ways to phyto-hormones of terrestrial plants (Craigie 201; Stirk et al. 2003).

In some places, the practice was of great importance to agriculture and contributed to improvement of poor soils, which are now used to produce valuable vegetables. However, seaweed availability is seasonal, its use requires intensive labour, and the composition of the material collected varies over time (Rayorath et al. 2008). This contributes to price oscillation and unpredictable variations in crop productivity.

As a result of these problems, seaweed use in agriculture declined with the development of chemical fertilizers and their increased availability at low prices. However, interest in the potential of seaweeds in improving soil fertility has renewed in recent years as a consequence of the environmental problems associated with chemical fertilizer use. Part of the excess fertilizer that reaches coastal waters is incorporated into seaweeds, so their collection and application can return nutrients to the soil (Kuperin et al. 2013), thus contributing to a zero waste agriculture.

Traditionally, before application of seaweeds to soil, they had to be sun dried and then washed to remove excess salt, which could lead to soil salinization, affecting productivity of crops, particularly those which are more sensitive to salt stress such as legumes. Recently several liquid fertilizers based on processed seaweed, mainly from the genus *Fucus*, have emerged on the market. In organic farming, these products have been applied in foliar sprays as a source of nutrients, natural hormones and to stimulate the natural defences of cultivated plants against diseases (Ugarte et al. 2006).

We are now reaching a stage where we need to improve plant productivity, in order to increase food production without increasing the farming area. To achieve this, we are changing our concept of crop management. In order to maintain crop sustainability, it is necessary to consider the soil nutritional balance, and guarantee that suitable levels of nutrients are present in the soil and provided to crops. We are also understanding the importance of soil ecology and the microbial community

(bacteria, archaea, fungi, microfauna, algae) to the rhizosphere assemblage and plant productivity, defence and abiotic stress tolerance (Zodape et al. 2011). In this context, the use of seaweed in agriculture may regulate rhizospheric function and stimulate plant growth through interaction with nutrient acquisition and/or hormonal balance (Sponsel et al. 2010).

There is evidence that seaweed-based fertilizers promote plant hormone balance, influencing production and degradation of cytokines and auxins, nutrient use efficiency and photosynthetic activity, resulting in greater plant vigour and, consequently, improved plant growth. Seaweed extracts can also have a stimulating effect on plants, by promoting root formation, flowering and fertilization. Plant growth promotion due to the effect of seaweed is dependent on the plant species and growth conditions. There are reports of consistent increases in lettuce biomass, size of potato tubers, and carrot productivity (Sivasankari et al. 2006).

However, not much is known about the mechanisms by which seaweed extracts promote plant growth.

In this work, we show that fertilization with seaweed extracts promotes tomato tolerance to salinity, and that Zn is involved in the maintenance of the leaf N^+/K^+ balance.

MATERIAL AND METHODS

Plants were obtained from tomato (*Solanum lycopersicum*, cv money-maker) seeds. Three plants with two completely developed leaves were transplanted into 4 L blow-moulded pots filled with sandy soil and irrigated with Hewitt (1966) nutrient solution during 1 month under non-saline conditions. Sandy soil, pH 6.2, was collected from agricultural sites used for tomato cultivation in rotation with other crops. The seaweed used in this work was *Ascophyllum nodosum* obtained from a supplier to the biofertilizer industry. The algae were dried, powdered and 2.5 g were placed in vials containing 50 mL of distilled water. The vials were then autoclaved at 110°C for 30 min. After decantation, 10 mL of the supernatant were applied per pot at the time of sowing, and twice more at intervals of 30 days.

Plants were kept in a greenhouse with 25/20°C day/night temperatures, relative humidity 65-80%; and mid-day photon flux density 700-800 $\mu\text{mol m}^{-2} \text{s}^{-1}$. After this period, plants were submitted to salinity treatments (0, 50, and 100, 150 and 200 mM NaCl). In order to avoid osmotic shock, NaCl was added progressively in weekly steps of 50 mM day^{-1} (Hessini et al. 2012).

Plants were irrigated with nutrient solution ($\text{pH } 6.0 \pm 0.1$) every 3 days, then harvested and separated into leaves and roots at the end of the vegetative stage (60 days after the beginning of salt treatment). There were 6 replicates per treatment in a total of 30 pots.

Plants were collected between 10:00 a.m. and 12:00 p.m. Plant dry weight (DW) was determined after oven drying samples to constant weight at 60°C . Na^+ and Zn^+ concentrations in dried plant leaves were determined by Inductively Coupled Plasma (Laboratório de Ionómica del CEBAS-CSIC, Murcia, Spain), with 6 replicates.

RESULTS AND DISCUSSION

Plants are sessile organisms that face a range of abiotic stresses, which are increasing on a global scale. High salinity is one of the major abiotic stress factors that significantly reduce crop yields and productivity. High NaCl conditions adversely affect plant growth through increased osmolarity, ion toxicity (i.e., Na^+ , Cl^- , and SO_4^-), nutritional imbalance, and oxidative stress (Turkan and Demiral 2009). The main toxic effects of Na^+ include inhibition of enzyme activities and disruption of intracellular K^+/Na^+ homeostasis (Zhu 2002). To cope with high salinity, plants have developed a number of mechanisms (Jithesh and Wally 2012). Presently there is increasing interest in the use of naturally occurring ‘biostimulators’ for enhancing the growth of agricultural and horticultural crops. Bacteria, fungi and protozoa, as well as marine algae-based seaweed extracts, can produce or contain biostimulators (Kurepin et al. 2013) that improve plant tolerance to stress, including salinity (Figure 1). It is interesting that under control (no saline) or low salinity conditions there was no significant effect of the application of *A. nodosum* extracts. However, at 200 mM NaCl, *A. nodosum* treated plants were twice the size of untreated plants, and not much smaller than the controls. These results are in line with others showing that extracts of seaweeds, and in particular those of *A. nodosum*, can produce a stimulating effect on plant growth, promoting greater root formation, increased flowering and fertilization (Rayorath et al. 2008; Alam et al. 2014).

A. nodosum has been extensively used in agriculture as a plant biostimulant (Craigie 2011). *A. nodosum* extract, when applied to plants, stimulates shoot growth and branching (Temple and Bomke 1989), increases

lateral root development (Metting et al. 1990), and improves nutrient uptake (Yan 1993). It has also been reported to improve plants' tolerance to environmental stresses such as drought, salinity and frost (Nabati 1991; Nabati et al. 1994), and improve stress tolerance in sensitive crop plants. Studies on citrus, grapes, bermuda grass and Kentucky blue grass have demonstrated that *A. nodosum* extract improves abiotic stress tolerance (Zhang 1997; Zhang and Schmidt 1999; Fike et al. 2001). Several bioactive compounds, including betaines (such as -aminobutyric acid betaine, -aminovaleric acid betaine, laminine (N6, N6, N6-trimethyl lysine), and glycine-betaine have been detected in *A. nodosum* and in the commercial products of *A. nodosum* (Blunden et al. 1985). Therefore the mechanism by which *A. nodosum* extracts improves plant stress tolerance is complex and involves several parallel pathways.

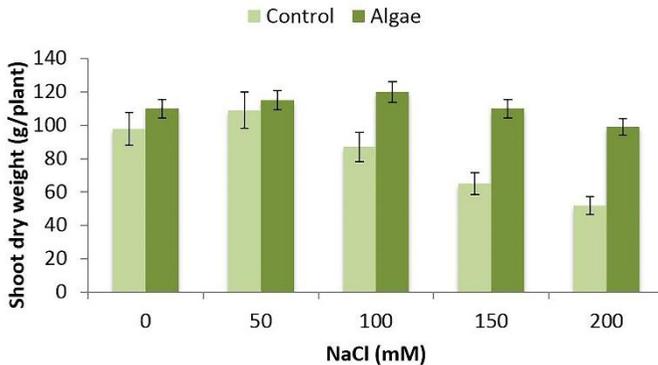


Figure 1. Biomass of plants (*Solanum lycopersicum*) not treated (Control) and treated with *A. nodosum* extracts (Algae) as a function of salinity. Bars represent mean values \pm standard deviation ($n = 6$).

Taking into consideration the mineral composition of the leaves at the end of the experiment (Figures 2-4), it was evident that salinity was associated with a decrease in leaf K^+ concentration, accompanied by an increase in leaf Na^+ concentration. This Na^+/K^+ imbalance has been reported as a cause of salinity damage. Since Na^+ competes with K^+ for binding sites and hampers metabolism by inactivating enzymes responsible for essential cellular functions (Munns and Tester 2008; Kronzucker and Britto 2011; Shahbaz et al. 2012). Plants treated with *A. nodosum* extracts displayed neither decreased leaf

K^+ concentrations, nor increased leaf Na^+ concentrations in response to salinity (Figure 2 and 3). Even under the highest $NaCl$ concentrations they maintained a Na^+/K^+ balance very similar to that of the control plants not exposed to salinity. The mechanism of entry of ions (including K^+ and Na^+) into the root space, xylem loading and unloading, overall Na^+ distribution and its compartmentalization in the plant system are dependent on transport proteins for ionic fluxes, which are guided by the electrical gradient and membrane potential across the membranes (Zhang et al. 2009; Craig and Moller 2010; Hauser and Horie 2010; Kronzucker and Britto 2011; Hedrich 2012).

A high extracellular Na^+ concentration increases the electrochemical gradient at the membrane, and thus favors passive transport. However, interpreting the effect of *A. nodosum* extracts is difficult, since there is currently little evidence of the direct involvement of a specific class of molecules in the regulation of ion selectivity in plants. Recent experiments with *A. nodosum* extracts suggest that their chemical components elicited endogenous biosynthesis of plant hormones (Rayorath et al. 2008; Wally et al. 2012). These hypotheses cannot be confirmed by our results, since hormonal analyses of the plants were not performed. However, based on the analysis of the leaf mineral components, one ion, Zn^{2+} , did display a pattern across treatments which was in line with those of K^+ and Na^+ (Figure 4).

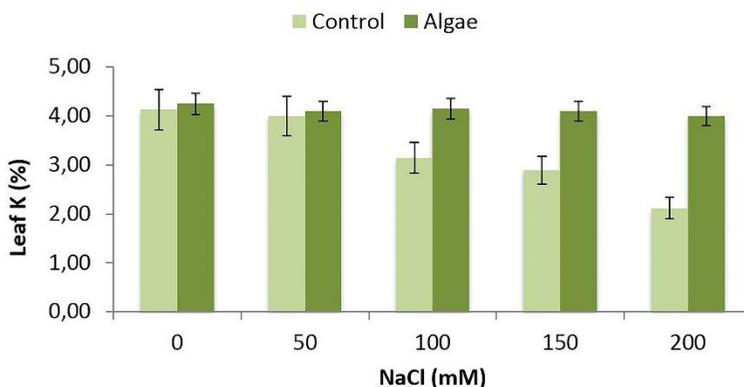


Figure 2. Potassium concentration in leaves of plants (*Solanum lycopersicum*) not treated (Control) and treated with *A. nodosum* extracts (Algae) as a function of salinity. Bars represent mean values \pm standard deviation ($n = 6$).

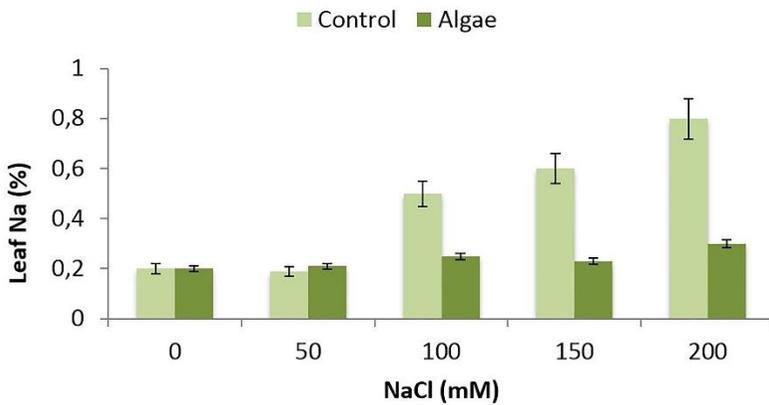


Figure 3. Sodium concentration in leaves of plants (*Solanum lycopersicum*) not treated (Control) and treated with *A. nodosum* extracts (Algae) as a function of salinity. Bars represent mean values \pm standard deviation ($n = 6$).

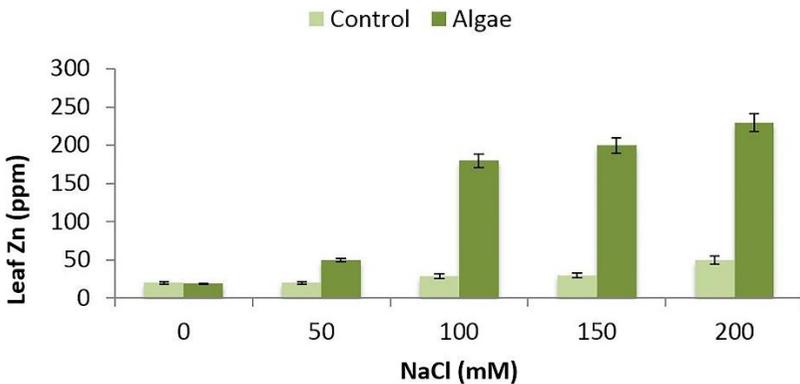


Figure 4. Zinc concentration in leaves of plants (*Solanum lycopersicum*) not treated (Control) and treated with *A. nodosum* extracts (Algae) as a function of salinity. Bars represent mean values \pm standard deviation ($n = 6$).

This result makes sense considering the physiological functions of Zn^{+} . As a common consequence of many environmental stresses, enzymatic antioxidants' responses to oxidative damage are activated in order to protect cell structures. Zn^{+} is a co-factor of many of the enzymes involved in the anti-oxidative stress, and it has even been suggested that Zn^{+} may develop cells' protection against ROS-induced damage (Marschner 1995; Cakmak, 2000).

CONCLUSION

The main question that remains to be answered is how *A. nodosum* extracts interact with Zn⁺ uptake and accumulation in leaves of tomato plants grown under saline conditions.

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