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# Electrical impedance spectroscopy: A tool to investigate the responses of one halophyte to different growth and stress conditions

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ABSTRACT

In order to evaluate the reliability and the practical use of the electrical impedance spectroscopy method applied in the area of salt resistance, electrical impedance spectra were compared in the leaves of halophyte plants cultivated under different growth conditions (biotope versus controlled conditions, hydroponic versus sand system cultures) and different salt stress conditions. The kinetic of impedance parameters was also monitored under short term salinity. The spectra of electrical impedance of leaves under biotope and laboratory conditions showed difference in the electrical response of *Cakile maritima* in the biotope and laboratory conditions. The response of electrical impedance parameters to salinity was also different in the hydroponic system when compared to the soil one, indicating more stressful conditions in solution culture. The amplitude of the curves of impedance spectrometry decreased when plants were stressed comparatively to their controls, with the highest electrical resistance in the presence of 50 and 100 mM while the lowest value was at 400 mM NaCl. The electrical resistance increased at an early stage after the application of salt stress reaching maximal value 180 min later, before it rapidly declined thereafter. The observed peak can translate a signal, that the plant could have received, which triggers a cascade of metabolic reactions allowing the plant to regain its hydro-ionic balance. In conclusion, electrical impedance spectroscopy can be used to quickly compare different growth conditions as well as different salinity treatments. This method can also separate between the osmotic and the ionic phases of the response to salt stress.

## 1. Introduction

Soil salinity has often been considered as a major constraint for agriculture. Nevertheless, the inherent plasticity in some native plants enables them to grow on such lands as reported for different halophytes. For these studies, different screening media such as hydroponics, sand or gravel culture and natural or artificially saline fields have been used. Information from these investigations provided clear indications on sensitivity or resistance of plants at germination, vegetative/generative stages, and at maturity (Tavakkoli et al., 2012). Resistance to salinity is not only a tolerance against $Na^+$ toxicity, but also a tolerance to water deficit and secondary stresses mainly nutrient deficiencies and oxidative damages (Munns et al., 2002). The oxidative stress has been considered as one of the most common and serious consequences of salinity that results in malfunctioning of the cellular membranes by increasing their permeability to ions and electrolytes. It was therefore considered worthwhile to test a salinity procedure that can collectively measure injurious effects of salts as well as of oxidative stress in order to assess the real salt tolerance potential and the factor(s) imparting resistance to a particular plant against these stresses. The measurements of reactive oxygen species (ROS), cell membrane stability (CMS) and lipid peroxidation are the main techniques that have often been used for screening against salinity tolerance in various crops such as wheat and barley (Sairam et al., 2002) and halophytes (Ellouzi et al., 2014). Recently, Labanowska et al. (2013) used electron paramagnetic resonance spectroscopy to measure membrane damages in drought stressed wheat plants. However, despite their many advantages, all these diagnostic methods were likely to be slow and destructive to the tissues, which could lead to erroneous interpretations. In addition, these tests were found to be markedly influenced by various experimental parameters and the nutritional of the tissues analyzed.

Electrical impedance spectroscopy (EIS) is another method to study the behavior and the properties of cell membranes in plants (Clausen and Dixon, 1986; Zhang and Willison, 1991; Mancuso, 1998). It is based on the study of the passive electrical properties

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of tissues, determined by the observation of the tissue electrical response to the injection of external electrical energy (Azzarello et al., 2006). In this method, alternating current (AC) is applied to a piece of plant tissues. Alternating current causes polarization and relaxation in the sample leading to changes in amplitude and phase of the applied AC signal. These changes are dependent on the tissue properties of the sample. At low frequency, the current flows in the apoplastic space of the tissues where ions are the main current carriers, which determine the total impedance. The symplastic space becomes conductive and at high frequencies, the symplastic and apoplastic resistance form a parallel circuitry (Repo et al., 2000)The impedance of the sample is formed of a real ($Z'$) and imaginary parts ($Z''$) in a complex plane where the impedance can be represented as a vector (arrow) of length $|Z|$. The angle between this vector and the $X$-axis is commonly called the phase angle (Fig. 1).

When the real and imaginary impedance were measured at different frequencies an impedance spectrum is obtained (Repo et al., 2000). Typically, the spectrum of plant tissues is composed of one or two arcs in the complex plane, depending on the sample under study and the range of frequencies used (Zhang and Willisson, 1992). Two types of equivalent electrical circuit models have been used to characterize plant tissues: lumped and distributed. In simple tissues such as potato tuber tissue, carrot roots and apple fruits, lumped electrical models, composed of a limited number of resistors and capacitors, may be used (Hayden et al., 1969; Zhang and Willison, 1991, 1993). In more complicated tissues such as woody stems, the tissue is better modelled by distributed circuits in which a reasonable number of unknown parameters may be estimated (Repo and Zhang, 1993).

The applications of electrical impedance measurement in plant tissues were numerous. In fact, this technique can be used to determine physiological conditions of plant tissues, particularly in relation to cold acclimation and freezing injury, (Zhang and Willison, 1992; Repo et al., 1994, 2000), heat injury (Zhang et al., 1993), heavy metals and flooding (Jócsák et al., 2010), ozone and $CO_2$ (Repo et al., 2004), drought (Wang and Zhang, 2010) and soil salinity (Mancuso and Rinaldelli, 1996). Another important application field is the *in situ* investigation of the size and activity of intact plant root systems in order to show the stress effects on the plant root growth (Ozier-Lafontaine and Bajazet, 2005; Cseresnyes et al., 2012, 2013; Ellis et al., 2013). Recently, Lin et al. (2012) developed an improved impedance measurement system that could help identify plant species and breeding systems in practices.

This method was applied in many tree plants like Scots pine (Repo et al., 2000), willow (Repo et al., 2005; Cao et al., 2011) and olive plants (Mancuso, 2000), but it has never been applied in halophytes. Halophytes are plants that naturally possess the traits needed to grow and reproduce on saline soils (Flowers and Colmer, 2008). By studying the mechanisms that halophytes use to deal with salinity, we might gain insight into which parameters are most promising to target for increasing salt tolerance in crop species. Most studies on halophytes used physiological, biochemical and molecular methods to quantify their responses to salt. Although some studies have been reported on the electrical responses of halophyte species under salinity stress (Shabala and Mackay, 2011), the electrical impedance spectroscopy method has never been used before to study the response of halophytes to salinity.

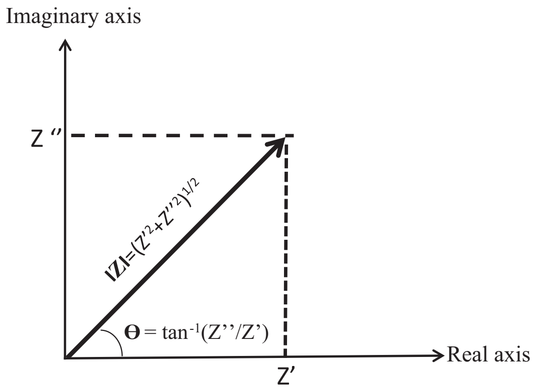

**Fig. 1.** Vector diagram of complex impedance. $Z$, impedance magnitude; $Z'$, real impedance, $Z''$ imaginary impedance, $\theta$, phase angle.

In the present experiment, we measured EIS parameters in the leaves of the coastal halophyte *Cakile maritima* cultivated under different growth conditions (biotope versus controlled conditions, hydroponic versus sand system cultures) and different salt stress conditions. The kinetic of impedance parameters was also monitored under short term salinity. We sought to find some parameters that change regularly with the salinity level and find one or several useful parameters that can be used as an index to assess salinity resistance of *C. maritima*.

## 2. Material and methods

### 2.1. Plant material

The plant material used in this study was sea rocket (*C. maritima* Scop.) from the Brassicaceae familiy. *C. maritima* is a C3 annual oilseed plant, growing spontaneously on sandy dunes along the Tunisian coasts. Several physiological and biochemical studies on *C. maritima* indicated the halophytic behavior of this plant that can survive up to 500 mM NaCl and accumulated salt ions preferentially in its leaves without dehydration and nutritional disorders (Debez et al., 2013). *C. maritima* was used in this study as a model of salt tolerant plant to learn about plant reactions that are not observed or experimentally reproduced in traditional glycophytic (or salt sensitive) models.

### 2.2. Culture conditions

#### 2.2.1. Experiment 1: biotope versus laboratory conditions

Analyses were performed on 10 plants collected in April 2014 from the biotope (*in situ* analysis), 10 plants from the biotope that were transplanted in the laboratory (acclimated plants) and 10 plants that were obtained from seeds and grown under laboratory conditions. All plants were cultivated in the soil from the beach of Soliman, near the Center of Biotechnology of Borj Cedria, at about 30 km N-E Tunis. It was a limono-sandy soil, the pH and the electrical conductivity of the aqueous extract (1/10) were 6.65 and 0.05 mmhos $cm^{-1}$ respectively. During the study, plants from the biotope were subjected to Mediterranean climate conditions. During the measurement days, plants were exposed to a maximum diurnal photon flux density (PFD) between 1300 and 1900 $\mu mol\ m^{-2}\ s^{-1}$. Maximum temperatures ranged between 23° and 31 °C. The leaves were sampled for electrical impedance analysis from plants at the beginning of the flowering stage; they were located at the fourth level, from the bottom of the stem.

#### 2.2.2. Experiment 2: Hydroponic versus sand cultures

Seedlings of *C. maritima* were separated in 2 lots; the first one (10 plants) was maintained in 2 l pots filled with an aerated saline nutrient solution (hydroponic culture). The plants of the second lot (10 plants) were cultivated in pots filled with sand, and irrigated regularly (each 2 days per week) with Hoagland nutrient solution (10 ml per irrigation) supplemented with NaCl (400 mM).