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A simple, fast and inexpensive method to assess salt stress tolerance of aerial plant part: Investigations in the mandarin group

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ABSTRACT

For grafted plants, salt stress tolerance of the aerial plant part is poorly documented. Thus, we developed a simple, fast and inexpensive method to identify tolerant genotypes. Twigs of 14 mandarin accessions that we previously analyzed as seedlings were cut in solution to prevent embolism and were then evaluated in salt stress condition for a week. Physiological parameters such as gas exchanges, leaf Cl^- and Na^+, as well as the presence of H_2O_2 and the activity of enzymes involved in ROS synthesis and detoxification processes were analyzed. One accession known to be tolerant as rootstock was shown to be sensitive with limited Cl^- translocation from the solution to the shoot while sensitive accessions when grown as seedlings presented limited wilting symptoms and accumulated large leaf Cl^- content. A model is proposed to explain the different strategies of the plant to cope with high toxic ion content. This method allows separation of the root compartment, where ion exclusion mechanisms may exist and have an impact on the salt stress tolerance of the whole plant.

1. Introduction

Soil salinity is a major environmental factor affecting citrus growth and productivity. In grafted trees, adaptation to abiotic stress such as salt stress is the result of an interaction between mechanisms in the root system but also in the aerial parts. The advantage of using rootstock is that it confers biotic and abiotic stress tolerance traits. In citrus, the *Poncirus trifoliata* genus confers tolerance to the citrus tristeza virus (CTV) but it is very sensitive to salt stress (Ferguson et al., 1990; Beattie and Revelant, 1992; Castle et al., 1993). 'Cleopatra' mandarin (*Citrus reshni* Hort. ex Tan.) is one of the most tolerant rootstocks used in the citrus industry (Moya et al., 2002) but it is susceptible to Phytophthora root rot, thus currently limiting its use. This cultivar is tolerant to CTV and is widely used in rootstock breeding programs in association with *Poncirus* to convey biotic and abiotic traits of tolerance. As in grape, salinity damage in citrus is usually associated with Cl^- accumulation but with Na^+ uptake (Banuls et al., 1997; Moya et al., 2003; Hussain et al., 2012; Fort et al., 2013; Hussain et al., 2014). *Poncirus* accessions are considered to be poor Cl^- excluders although they have a high capacity to exclude Na^+ at low salinity levels (Sykes, 2011), which partly explains the salt-sensitive nature of this rootstock (Saleh et al., 2008; Zekri and Parsons, 1992). According to Moya et al. (2003), if Cl^- absorption is not limited at the root level, ions will be passively translocated to the leaves via transpiration and will cause necrosis and eventually defoliation. When using 'Carrizo' citrange rootstock, which is a hybrid [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf], Cl^- root-to-shoot translocation occurs in leaves of seedlings (Romero-Aranda et al., 1998) but also in leaves of grafted varieties (Walker et al., 1983). Accumulation of Cl^- and Na^+ ions to toxic levels is associated with a decrease in the osmotic potential and with nutritional imbalances (Byrt and Munns, 2008), leading to a reduction in plant growth and fruit yield (Storey and Walker, 1998).

The osmotic potential of plants is regulated after stomata closure and subsequent limitation of photosynthesis, which involves several processes such as K^+ uptake, Na^+ and Cl^- compartmentalization outside of the cytoplasm and synthesis of compatible solutes (Ashraf, 1994). In addition to salt stress, a secondary effect

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Table 1
List of mandarin accessions subjected to 7 days of salt stress, as well as associated symptoms observed at the end of the experiment.

Mandarin common name	Abbrev.	Tanaka classification	Swingle and rice classification	SRA[a]	Intensity of symptoms	Symptoms
'Vietnam à peau fine'	Viet	*C. kinokuni* Hort.	*C. tachibana*	766	–	No symptom
'Dancy'	Danc	*C. tangerina* Hort.	*C. reticulata*	552	*	Very low apparent symptoms
'Shekwasha'	Shek	*C. depressa* Hayata	*C. reticulata* hybrid	982	*	Slight wilting of leaves
'King of Siam'	KiOS	*C. nobilis* L.	*C. reticulata* hybrid	273	**	Leaf curling
'Bintangor Sarawak'	BiSa	Bintangor (C. reticulata Blanco × *C. aurantium* L.)	*C. reticulata* Blanco	683	***	Strong leaf curling
'San Hu Hong Chu'	SHHC	*C. erythrosa* Hort.	*C. tachibana*	769	*	Very low apparent symptoms
'Sun Chu Sha'	SuCS	*C. reticulata* Blanco	*C. reticulata* Blanco	786	*	Slight wilting of leaves
'Cleopatra'	Cleo	*C. reshni* Hort.	*C. reticulate* var. austera	948	***	Wilting and curling of leaves
'Ponkan'	Ponk	*C.reticulata* Blanco	*C. reticulata* Blanco	584	****	Strong curling, drying and wilted leaves
'Anana'	Anan	*C.reticulata* Blanco	*C. reticulata* Blanco	652	**	Wilted and curling of leaves
'Fuzhu'	Fuzh	*C.erythrosa* Hort.	*C. tachibana*	775	**	Strong leaf curling with slight wilted tips of leaves
'Beauty'	Beau	*C.tangerina* Hort.	*C. reticulata*	411	–	No symptom
'Willowleaf'	WilLe	*C.deliciosa* Ten.	*C. reticulata*	133	*	No leaf curling, leaves slightly wilted
'Nasnaran'	Nasn	*C.amblycarpa (Hask.)* Ochse	*C. reticulata* hybrid	896	–	No symptom

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of the presence of toxic ions is the triggering of oxidative stress (Gueta-Dahan, 1997; Arbona et al., 2003; Tanou et al., 2009), which causes damage to the leaf photosynthetic machinery via ROS overproduction such H_2O_2(Gueta-Dahan et al., 1997; Arbona et al., 2003; Tanou et al., 2009). Plants have evolved efficient antioxidant systems that can protect them from the damaging effects of oxidative stress (Asada, 1999). Among them, antioxidant enzymes are involved in H_2O_2 formation from superoxide ($O_2{}^-$) such as superoxide dismutase (SOD, EC 1.15.1.1) or H_2O_2 scavenging such catalase (CAT, EC 1.11.1.6) and peroxydase (POD, EC 1.11.1.7) can be cited.

Mandarin germplasm was classified as *Citrus reticulata* Blanco by Mabberley (1997), while Swingle and Reece (1967) classified it in *Citrus reticulata* and *Citrus tachibana* (Mak.) Hodgson (1967) divided mandarins into four species, including *Citrus unshiu* (satsumas), *Citrus reticulata* ('Ponkan', 'Dancy', Clementines), *Citrus deliciosa* ('Willowleaf') and *Citrus nobilis* ('King'). However, Tanaka (1954) divided mandarins and related species into five groups that included 36 species using morphological differences in the tree, leaves, flowers and fruits. Using this classification, we investigated 14 different mandarin seedling accessions when subjected to salt stress (Ben Yahmed et al., 2015). Tolerance traits we characterized were related to root-to-shoot Cl^- translocation limitation and leaf detoxification processes. From an agronomic standpoint, mandarins can be roughly clustered into two main groups: (i) one group that includes accessions used as rootstock and produce fruit with an acidic taste, such as *Citrus reshni* Hort. ex Tan. ('Cleopatra') or *Citrus depressa* Hayata ('Shekwasha') and (ii) varieties selected for fruit quality that are grown grafted onto a rootstock. In our hands, the first group was found to be tolerant, with limited root-to-shoot Cl^- translocation while some varieties were also quite tolerant even though they had accumulated high Cl^- contents (Ben Yahmed et al., 2015).

For a seedling, little is known regarding its tolerance to salt stress independently of the capacity of the root to limit the root-to-shoot translocation of toxic ions. Seedlings of accessions that may be poor root Cl^- excluders may thus have developed more efficient mechanisms in the leaves to cope with the presence of toxic ions. On the contrary, accessions that exclude Cl^- in the roots and thus are seldom affected by a high Cl^- content in the leaves may not have developed such mechanisms.

Salt stress experiments were previously assessed using citrus plants cultivated in vitro without a root system (Montoliu et al., 2009). This simple and fast method showed that shoots accumulated similar levels of Cl^- when cultured without roots and exhibited similar leaf damage. However, this method requires to propagate in vitro the plant material. Thus, culture conditions (e.g.,: saturated humidity) may induce a specific behavior in salt stress condition. We used another approach in order to characterize the salt tolerance potential of the aerial part in different mandarin accessions. Twigs were cut and were plunged in a saline solution for a week so as to evaluate the physiological parameters. According to the cohesion tension theory (Jackson et al., 1994), the water transport system in plants is based on water columns transmitting negative pressure to xylem vessels and finally to the soil. Air spaces that may be present in the xylem can destabilize the water columns and consequently induce breakage of hydrogen bonds between water molecules, leading to embolism (Tyree et al., 1994). In order to avoid embolism twigs were cut in solution allowing to maintain the water column as well as gas leaf exchanges throughout the experiment without inducing embolism. This simple method allowed us: (1) to separate the root compartment where ion exclusion mechanisms may exist when assessing seedlings, (2) to promote fast absorption of ions in the transpiration stream through the xylem vessels to the leaves, and 3) to get a prompt response regarding the phenotype induced at the leaf level in investigated scions.

2. Materials and methods

2.1. Plant material and salt stress application

Experiments were carried out on 14 2-year-old mandarin accessions (Table 1) grown in greenhouse conditions (Ben Yahmed et al., 2015). Nine twigs (50–60 cm) from nine independent plants of each accession were used. For each accession, twigs had a similar size and number of leaves. Twig extremities were cut a second time (1 cm from the tip) under solution and placed in 60 mL glass test tubes (2.5 cm diameter). Three twigs of each accession were assigned as controls (water) and the six were assigned to undergo a 50 mM salt stress. The initial solution volume in the tube was 50 mL. Tubes were then kept sealed around the twig with parafilm hinder direct water evaporation. Tubes with twigs were maintained in greenhouse conditions for a week under natural photoperiod conditions, with day/night temperatures of 25–32 °C/18–22 °C, respectively, and a relative humidity ranging from 60 to 85%. The tubes were weighted daily to measure the volume of solution absorbed. The amount of consumed water was measured daily until the end of the experiment. When required, water was added in tubes for controls to maintain the twig extremities in solution.