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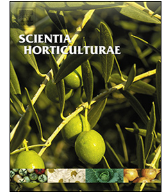

Evaluation of salt resistance mechanisms of grapevine hybrid rootstocks

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ABSTRACT

The problem of soil salinization is increasing critically in China. The widespread rootstock Beta (*V. labrusca* × *V. riparia*) is relatively weak (Du et al., 2015), and the commonly used phylloxera-resistance rootstocks were still unable to satisfy the needs of production as well. F1 hybrids A15 and A17 of *V. amurensis* Rupr. cv. Zuoshan-1 × SO4 (*V. berlandieri* × *V. riparia*) had strong salt tolerance were irrigated with 100 mmol·L^{-1} NaCl to explore the salt resistance mechanisms, the male parent SO4 was used as reference. The result showed that root activity was higher under salinity conditions in A15 and A17, with a more complete chloroplast structure investigated than that of SO4 under the NaCl treatment. Na^+ accumulations in leaves and the whole plant of A15 and A17 were lower than SO4, whereas the Na^+ retention capacity in roots were higher than SO4. The total amount of Na^+ and K^+ of roots and aboveground portion of grafted plants A15/SO4, SO4/A15, A17/SO4, and SO4/A17 further confirmed that stronger root Na^+ retention capacity and upward transport of K^+ of A15 and A17 under NaCl treatment. The non-invasive micro-test technology (NMT) manifested that A15 and A17 had a higher Na^+ exclusion ability and a better root K^+ retention ability; salt stress significantly up-regulated the relative expression of *VvNHXP* and *VvHKT* family genes in roots of A15 and A17.

1. Introduction

High concentrations of salts in soils is a global challengethat restricts agricultural development and crop growth (Shrivastava and Kumar, 2015). Over 950 million hectares of agricultural land in global are subject to soil salinity (Rengasamy, 2010), among them, approximately 36 million hectares in China (Li et al., 2005; Wang et al., 2011), which account for about 1/3 of the total saline-alkaline land in Asian.

Among all types of salinity, the most dissolvable and extensive salt is NaCl, and Na^+ poisonousness therefore prevails in the most natural production place affecting plant growth. The detrimental effects of high salt result from both a water deficit caused by osmotic stress and the influence of excess Na^+ ions on key biochemical processes (Zhang and Blumwald, 2001).Accordingly, previous studies have demonstrated that use of rootstocks with higher salt tolerance can improve salt tolerance when they encounter salinized soils (Fisarakis et al., 2001; Paranychianakis and Angelakis, 2008; Verma et al., 2010). Numerous studies on plant salt resisitance have indicated the existence of plenty differences in stress response amongst varieties within grape species and have shown that genetic variability amongst species is an important basis for exploring salt resistance mechanisms (Munns et al., 2000). Thus, to research grapevine rootstock strategies against high salinity is of fundamental significance. Plants utilize multiple biochemical and molecular coping strategies, including selective buildup or exclusion of salt ions (Parvaiz and Satyawati, 2008), control of ion uptake by the roots and transport into the leaves (Greenway and Munns, 2003), ion compartmentalization (Lv et al., 2012), synthesis of compatible osmolytes (Tester and Davenport, 2003), alteration to the photosynthetic pathway (Sudhir and Murthy, 2004), changes in the membrane structure (Guimarães et al., 2011), induction of antioxidative enzymes (Apen and Hirt, 2004), stimulation of phyto-hormones (Upreti et al., 2012), and regulation of gene expression (Parida and Das, 2005).All in all, maintaining cellular and whole-plant K^+/Na^+ homeostasis is necessary for plant adaption to NaCl stress (Sun et al., 2009; Chen and Polle, 2010), the salt tolerance therefore relies on the capacity of plants to restrict the net transport of Na^+ from the root to the shoot (Niu et al., 2018). This process depends on some pivotal mechanisms; one of which is the adequate Na^+ efflux from the root to the outside medium. The plasma membrane-located H^+-ATPase and Na^+/H^+ antiporters are ubiquitous membrane proteins that play crucial roles in cellular pH and Na^+ homeostasis throughout the biological kingdom (Shi and Zhu, 2002; Zhu, 2016). Plants remove excess Na^+ from the cytoplasm by either relegating it to the apoplasts or compartmentalizing it to the vacuole by Na^+/H^+ anti-porters associated with the plasma membrane

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or vacuolar membrane (Hasegawa et al., 2000; Blumwald, 2000). Northern arid, semi-arid and semi-humid areas which hold the majority of salt and alkaline, are the most important wine grape-producing zones, where the problem of soil salinization is increasing critically. Many studies showed that rootstocks had positive effect on salt tolerance of grapevine, the Flame seedless vines raised on 110R witnessed low Na^+ content and Na^+ :K^+ ratio, and maintained high leaf water potential and osmotic potential and root: shoot dry mass ratio (Upreti et al., 2012), Ramsey rootstock improved the salt tolerance of Sultana under saline field conditions (Walker et al., 1997), rootstocks Ramsey, 1103 Paulsen, 140 Ruggeri had good effects on grape berry development, ion concentrations and soluble solids within the Murray- Darling viticultural region with an irrigation water salinity of either 0.43 dS/m (low salinity site) or 2.3 dS/m (high salinity site) (Walker et al., 2000; 2002; 2004). Beta (*V. labrusca* × *V. riparia*) has the widespread use as rootstock, but the salt tolerance of Beta is relatively weak (Du et al., 2015). Among the commonly used phylloxera-resistance rootstocks, only 1103 P, (*V. berlandieri* × *V. rupestris*) has strong salt tolerance (Galet, 1988). However, its salt tolerance is still unable to satisfy the needs of production as well. In Europe, the main goal of grapevine rootstock breeding is to resist phylloxera, and the second goal is to resist ecological adversity. In China, the main source of ecological adversity is salt. In 2010, we used hybrids of Zuoshan1♀×SO4♂ to evaluate the phylloxera and cold hardiness (Du et al., 2017), and then the rootstocks with high phylloxera resistance and greater cold resistance than Beta were used for evaluation of salt resistance (Fu et al., 2017), and the results showed A15 and A17 had strong salt tolerance. We selected A15 and A17 to explore the salt resistance mechanisms under salinity stress.

2. Materials and methods

2.1. Grapevine tissue culture seedlings as test material

2.1.1. Growth and treatment conditions

One-month old, consistent tissue culture seedlings of A15, A17, and SO4 were used as materials. The Murashige and Skoog (MS) culture medium of secondary culture was completely washed off, and the seedlings were transferred to a new cultureflask(oneplantperbottle),andtheculturesolution(withoutsucrose compared with MS medium) was refreshed every day. Plants were placed in a tissue culture room with ambient temperatures of 23–25 °C and 16–18 °C for day and night, respectively. Light was supplied by sodium lamps with a 14-hour photoperiod (photon flux density of 400 μmol m^{-2}·s^{-1}). Seedlings were precultured for three days to allow them to acclimate to the new conditions. After three days of preincubate, half of the seedlings were treated with MS liquid medium containing 100 mmol·L^{-1} NaCl, and the control was simply treated with an equal volume of MS liquid medium. Each treatment contained three replicates of 30 plants each. Seedlings were treated for five days, then leaves or roots were sampled to measure the leaf lipid peroxidation, electrical conductivity, and root activity. In addition, at hours 0, 12, 24, 36 and 48, roots were sampled and promptly frozen in liquid nitrogen and stored at −80 °C for the testing of *VvNHXP* and *VvHKT* family genes expression; fresh leaves were harvested to examine the ultrastructure of chloroplasts after 0 and 36 h of treatment.

2.1.2. Assays of membrane stability and root activity

After 5 days of salt treatment, leaves and roots were collected for analysis. The level of lipid peroxidation of the leaves was estimated by measuring their peroxidation products, thiobarbituric acid reactive substances (TBARS), according to Liu et al. (2015). Relative electric conductivity of the leaves was determined according to the method described by Zhao et al. (2002). In addition, root activity was detected by the triphenyl tetrazolium chloride (TTC) method, and it was represented as the deoxidization ability (mg·g^{-1} h^{-1} FW; Zhao et al., 2002).

2.1.3. Observation of chloroplast ultrastructure

The ultrastructure of chloroplasts was observed on the basis of Shu et al. (2013). Leaf segments, which were excised from the six leaves of control and NaCl treatment, were soaked in a stationary liquid (2.5% glutaraldehyde; pH = 6.8) for 24 h, and then rinsed with 0.1 mol·L^{-1} phosphate buffer (pH = 6.8) at 4 °C. They were dehydrated in a series of graded ethanol with 50%, 70%, 95%, and 100%, followed by transfer to 100% acetone at room temperature. Finally, the samples were embedded with the LR white resin and polymerized at 55 °C for 24 h, ultrathin sections were cut and double stained with a diamond knife, and then observed using transmission electron microscopy (TEM) and photographed using a charge-coupled device (CCD) camera.

2.1.4. Total RNA extraction and gene expression analysis

The total RNA was extracted from root tissues according to the method of the Trizol reagent (TianGen Biotech, Inc., Beijing, China), in which RNase-free DNase I was added at 37 °C for 15 min to remove any contaminating genomic DNA prior to cDNA synthesis. The RNA was purified with RNeasy Kit (TianGen Biotech, Inc., Beijing, China) according to the manufacturer's instructions and was quantified by

spectrophotometry, the OD_{260} / OD_{280} of RNA extracted was 2.0–2.2, which indicated that the purity of the RNA was high (a ratio of 2.0 was considered a mark of high quality RNA). The quality and integrity of the RNA were detected by electrophoresis through agarose gels stained with ethidium bromide as well. Primers were designed by the company of Sangon Biotech in China, and the primer sequences are shown in Table 1, the qRT-PCR was performed using the PrimeScript® reverse transcription kit according to the manufacturer's instructions (TaKaRa Biotechnology, Dalian, China); the real-time fluorescent dye was developed using the UItraSYBR Mixture (Low ROX) kit. The 20 μL mixcure consisted of 10 μL of 2 × UItraSYBR Mixture (Low ROX), 8 μL of ddH_2O, 0.5 μL of forward primer, 0.5 μL of reverse primer, and 1 μL of cDNA template at 100 ng·$μL^{-1}$. The transcripts of the *VvUBIRQ* gene were used to standardize the cDNA samples for different genes, the relative expression level of the gene was calculated using the $2^{-\triangle\triangle CT}$ method of Livak et al. (Livak and Schmittgen, 2001). These qRT-PCR experiments were repeated three times, based on three separate RNA extracts from three samples.

2.1.5. Measurement of Na^+ and K^+ fluxes in roots with NMT

The non-invasive micro-test technology (NMT) technique (Younger USA LLC, Amherst, MA, USA) was used to determine the net Na^+ and K^+ fluxes in roots (Fig. 1) of grape plantlets in vitro (Lang et al., 2014). A large number of previous studies have shown that high concentrations of Na^+ will seriously affect the signal-to-noise ratio of Na^+; in other words, Na^+ electrode is not suitable for determination of high Na^+ concentration solution. Therefore, in this study, we did not measure changes of real-time Na^+ flux in the root zone of three grape strains under NaCl stress, but rather we compared the changes within the apical meristematic zone of each grape strain after NaCl stress was relieved. Similar size and site of roots of tissue culture seedlings of A15, A17, and SO4 were treated with 100 mmol·L^{-1} NaCl for 24 h, leading to

Table 1
Gene-specific primers designed for qRT-PCR.

Gene	Forward primer	Reverse primer
VvUBIRQ	TCTCAACCCAAAGGCTAATC	GCATAGAGGGAAAGAACAGC
VvNHXP	GGTGTTTCCCTACAGAGCG	CAAAGCGACGGTGTAAGG
VvHKT1	GAGCATCGCCCTGGAAGTC	TGCCGAGAACAGTGATACCC
VvHKT2	ATTTTCAAGAAGAACTCGGG	TTCCCCAGAACCCAGATGAC
VvHKT3	TGCGGTGGAAATGGAGGTT	AGGCCAAGCATGGAAGTGAA
VvHKT4	CTTGTTTCCCTCCTGCCT	CAGAATGAAGCCCAGAAC
VvHKT5	CATCTTGGTGTTAGTCGTCGTA	CTTTTTCCTCTCCGTTATGC

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