Study on Effects of salt stress on the Suberin Lamella of grapevine roots

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Abstract. Grape is one of the oldest tree species in the world which have a relatively high tolerance to salt stress. The function of Suberin Lamella is to control the transport of water and ions, which has a positive effect on salt tolerance. However, whether the suberin lamella of grape root is related to its salt-tolerance has not been revealed. In this study, suberin lamella in roots of two grape varieties, "Crimson seedless" and "1103p", were stained by FY0888. Results showed that salt stress induced the appearance and thickening of suberin lamella of grape root cortex. The induction effect was very obvious in salt-toerant "Crimson seedless", while the effect was weak in "1103P", indicating that the suberin lamella of grape was indeed involved in the salt tolerance of grape.

Keywords: Grape, Suberin Lamella, Salt tolerance, Salt exclusion.

1. Introduction

Suberin lamella is a substance consisting of fatty acids and glycerol, which acts as a protective barrier between the plants and environment. The function of it is controling the transport of water and ions into stele [1, 2]. Abiotic stresses, such as salt stress, could induce the thinken of suberin lamella in root of plants. The induction was found in many species such as Arabidopsis and rice [3, 4], indicating that suberin lamella in plants had a positive effect on salt tolerance.

The observation of suberin lamella is the main method to study it, which includes the thickness, integrity and location of formation. FY088 and Sudan Red 7B are the most commonly used dyes. After FY088 staining, yellow fluorescence could be observed of suberin lamella. While, Sudan red 7B is a non-fluorescent dye, and the suberin lamella could be specifically dyed into red [5].

Eurasian cultivars are generally considered to be more salt-tolerant due to their place of origin. Among them, *Vitis vinifera* "Crimson Seedless" is widely planted in northwest China and Xinjiang Province of China. The grape stock 1103 Paulsen (1103P) has less salt tolerance than " Crimson Seedless "[6]. However, whether the suberin lamella r of grape root is related to their salt exclusion ability still needs further study.

2. Material and Method

2.1 Materials

Vitis vinifera Crimson Seedless and 1103P grape tissue culture seedlings were used as the materials in this study. The incubation conditions were 16h light/dark for 8h, light intensity of 110 umol · $m^{-2}S^{-1}$, and light temperature of 25℃. When the seedling height reaches 10cm, roots were carefully removed from the medium and immersed directly into 0, 50 mM NaCl of liquid Hoagland medium for 48h. Three replicates were set for each treatment. After the salt treatment, the roots were removed and the roots of the tissue culture seedlings were washed with pure water three times. Three seedlings with consistent growth were selected for two varieties and two treatments.

2.2 Root Section

The root was cut off from the grape seedling. Then the root segment 45 mm from the root tip was cut and then placed on the potato block. Mark both ends of the root, then cut through the potato block in order to get the 40 mm, 30 mm and 20 mm from the root tip, respectively.

2.3 Staining for Observation

The suberin lamella showed yellow fluorescence dyed by FY088 at 365 nm according to Krishnamurthy (2009)[3]. The FY088 dye was added to saturated lactic acid and dissolved in a 70℃ water bath for 1 h in 0.01% (w/v) FY088 dye solution. Drop dye onto sections for 1 hour

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staining (protected from light). Distilled water was washed three times and observed using a fluorescence microscope.

3. Results

3.1 Effect of salt Stress on Suberin Lamella of Crimson Seedless

After staining, different degrees of yellow fluorescence appeared in both Crimson Seedless and 1103P roots and showed significant differences before and after salt treatment (Figure 1 and Figure 2).

In the control group of Crimson Seedless, the fluorescence of the suberin lamella was not observed at 20 mm from the root tip (Figure 1 A). While, the suberin lamella started in the outer cortex 30 mm from the root tip (Figur 1C), and weak fluorescence remained 40 mm from the root tip (Figure1 E). In the 50 mM NaCl treated group, the fluorescence of the suberin lamella was observed 20 mm from the root tip, and the suberin lamella was almost covered by the entire outer tical area (Figure1 B). This fluorescence intensity gradually increased with the distance from the root tip (Figure1 D, F).

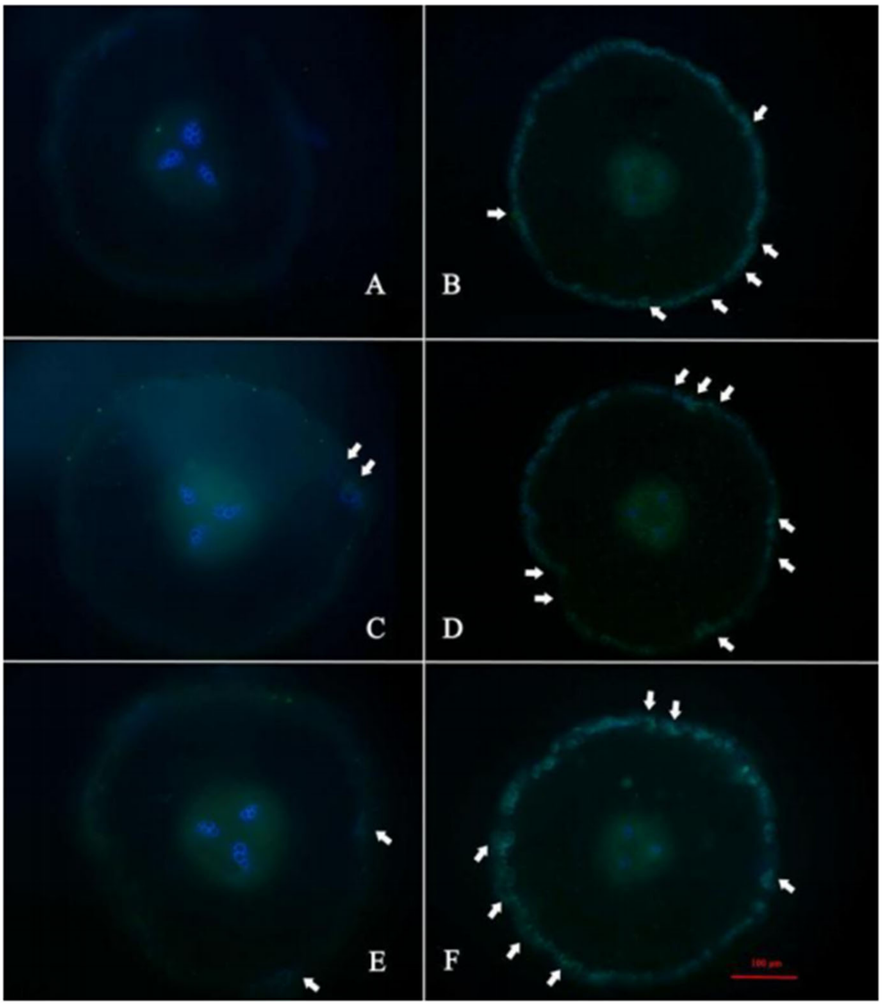

Fig. 1 Effect of 0 mM and 50 mM NaCl treatment on Crimson Seedless suberin lamellae of grape varieties. A, C and E were 20 mm, 30 mm and 40 mm sections respectively, and B, D and F were 20 mm, 30 mm and 40 mm sections, respectively

3.2 Effect of salt Stress on Suberin Lamella of 1103P roots.

A small amount of suberin lamellae fluorescence could be observed in the salt-sensitive variety 1103P control group (Figure 2 A). As the distance from the root tip increased, suberin lamellae fluorescence was detected in more outer cortex cells (Figure 2C, E). After 50 mM NaCl treatment, the suberin lamellae appeared earlier and the fluorescence was stronger than the control group, but this enhancement was much less significant than in Crimson Seedless (Figure2). These results showed that salt stress induced the appearance and thickening of the suberin lamellae in the outer cortex, which was evident in CrimsonSeedless and weak in 1103P.

The thickening of the suberin lamellae in grape roots may be induced by salt stress, and the role of thickening was more pronounced in Crimson Seedless, while the fluorescence was weaker in 1103P, indicating that nacl treatment did thicken the embolastic layer after salt stress.

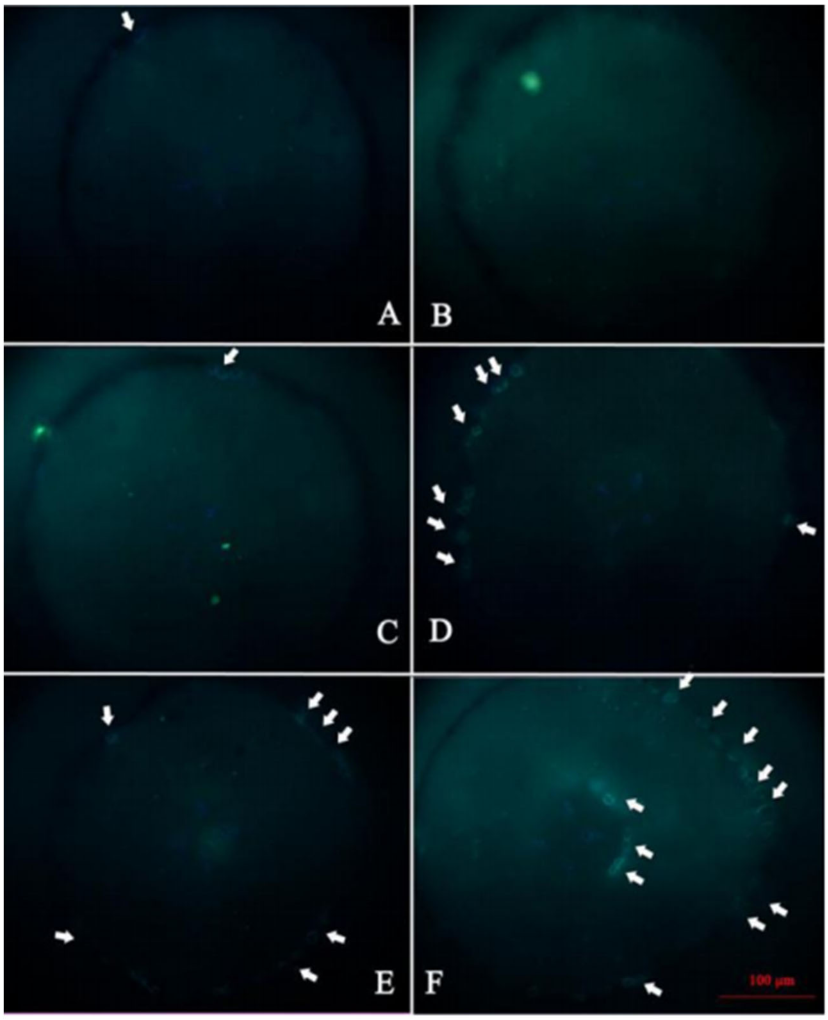

Fig. 2 Effect of 0 mM and 50 mM NaCl treatment on 1103P suberin lamellae of grape varieties A, C and E were 20 mm, 30 mm and 40 mm sections respectively, and B, D and F were 20 mm, 30 mm and 40 mm sections respectively

4. Discussion

Suberin lamellae can resist plants to stress because it is a direct interface between plants and soil [2, 8]. The salt tolerance studies in rice show that suberin lamellae exerts salt tolerance through its own thickening [3, 9]. As shown in Figure 1 and Figure 2, the root cortex of CrimsonSeedless and 1103P grape varieties were observed to show suberin fluorescence. In addition, the fluorescence morphology changed from a ring covering the entire radial wall. This phenomenon showed that the salt resistance of grapes can be tested by the thickening of suberin lamellae. This indicated that the suberin lamellae of grape root is indeed involved in the salt tolerance.

In addition, salt stress-induced thickening of the suberin lamellae was more pronounced in Crimson Seedless, which was slightly weaker in 1103P. Previous results showed that Crimson Seedless had significantly higher salt tolerance than 1103P [10] under NaCl treatment. Therefore, we speculated that the difference in suberin lamellae changes directly led to the difference in salt tolerance between the two grape varieties.

Acknowledgement

We are grateful for financial support from Shandong Province.the Natural Science Foundation of Shandong Province (ZR2020MC140, ZR2019BC114), Shandong Modern Agricultural Industry Technology System (SDAIT-06-14) and the Basic Research Projects of Integration of Science, Education and Industry of Qilu University of Technology (Shandong Academy of Sciences) (2022PX033).

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