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Relationship between rhizosphere soil properties and blossom-end rot of tomatoes in coastal saline-alkali land

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ABSTRACT

Blossom-end rot (BER), one of the common physiological disorders in tomato production, decreases fruit crop yields and quality, resulting in economic losses. In this study, five cultivars of tomatoes were studied in saline soil and the microbial diversity and richness in the rhizosphere and bulk soil were compared in diseased and healthy plants. The salt content was generally lower in the rhizosphere compared with bulk soil. There was a significant positive correlation between salinity and BER incidence. In the rhizosphere soil, salinity had little effect on catalase and invertase activity, but had a significant positive correlation with phosphatase activity, which was also positively correlated with the incidence of BER. Among all the samples, the highest relative abundance of *Proteobacteria* was in soil in which cultivar Mengyu grew; this cultivar was the healthiest (lowest BER) among the five cultivars tested. The incidence rate of tomato blossom-end rot had a significant negative correlation with the relative abundance of soil microorganisms, but was positively correlated with abundance of *Bacteroidetes*. The result suggested that the occurrence of tomato blossom-end rot was influenced by soil chemical (salinity), biochemical (phosphatase activity) and biological properties (relative abundance of specific bacterial phyla).

1. Introduction

Lycopersicon esculentum Mill, family *Solanaceae*, is an important food source (Douglas, 2017). It contains relatively large amounts of antioxidative substances that can reduce oxidative damage to various macromolecules, including lipids, proteins and DNA (Martínez-Madrid et al., 2007; Li et al., 2016).

Blossom-end rot (BER) is a common physiological disorder in tomato that causes large economic losses in greenhouse- or field-grown tomatoes (Suzuki et al., 2003). Tomatoes suffering from the disorder often shed early and have unacceptable quality (Zhang, 2003). Physiological disorders are not linked to pathogenic microorganisms; instead, they are usually caused by inappropriate living environment, such as poor soil physical and chemical properties, excess or insufficient nutrition, and poor climatic conditions such as high relative humidity (Cornell, 2015; Mi, 2012). Blossom-end root usually starts around umbilicus, with the tissue becoming soaked dark green; after about a week, that part of fruit becomes dark brown and rotten (Dong et al., 2001). One of the first visual symptoms is membrane rupture (Suzuki et al., 2003); it should be borne in mind that relatively poor supply of calcium, which has an important role in the stability of the plasma membrane as well as cell wall (White and Broadley, 2003), is frequently associated with blossom end-rot in tomato.

Soil enzyme sources comprise secretions of soil animals, plants and microorganisms. These enzymes catalyze soil nutrient cycling. Enzyme activity is not only one of the indicators of biological activity and thus soil quality and soil fertility (Alkorta et al., 2003), but also can play a role in promoting benign ecological cycles in soil and preventing pests and diseases (Wang et al., 2010; Badiane et al., 2001). The most studied enzymes are: invertase, urease, catalase, dehydrogenase, phosphatase (acid, neutral, alkaline), and cellulolytic enzymes. Soil enzymatic activity is affected by plant rhizosphere secretions, and also by soil salinity and pH. Additionally, the higher the salt content in soil, the higher the prevalence of blossom-end rot (Zhai et al., 2015).

Catalase acts as oxidoreductase, decomposing hydrogen peroxide. In low concentrations, hydrogen peroxide helps plants resist the adverse growth environments, such as temperature extremes, water shortage, salinity, diseases, herbicides, and various biotic stresses (Bandick and Dick, 1999). However, in high concentrations, hydrogen peroxide is linked with oxidative stress and a range of deleterious effects on living cells. Invertase decomposes sucrose to its components glucose and fructose that serve as nutrient and energy sources for all living

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organisms. Given that invertase activity increases as soil maturity increased, the strength of soil invertase activity may indicate a degree of soil maturation and fertility. Phosphatases could converted many forms of organic phosphorus in soil into available inorganic phosphorus. Phosphatases could be used as an indicator of the direction and intensity of phosphorus biotransformations in soils (Chen et al., 1993). Based on the optimum pH, phosphatases are divided into acid, neutral and alkaline phosphatases, with optimum pHs around 5.0, 7.0 and 10.0, respectively.

The structure and activity of soil microbial community are not only important to the biogeochemical cycles in soil, but also play an important role in the process of organic matter decomposition. These microbial parameters characterize soil fertility and soil environmental quality in addition to the soil enzymatic activity indicators (Zelles, 1999). Root exudates can provide not only nutrients to the rhizosphere microorganisms, but some exudates could also act as signalling molecules to regulate the composition and activity of the rhizosphere microbiome. In addition, rhizosphere microorganisms could make nutrients available and promote root absorption, playing an important role in plant growth and health (Song et al., 2011). Moreover, changes in the abundance, activity and community structure of rhizosphere microbes can influence plant resistance to adverse environmental conditions, such as BER, and pathogen invasion (Schippers et al., 1987).

Our previous study on the content of elements (K, Ca, Na, Mg, P, and Zn) showed that there was no significant difference in soil and tomato plant organs contents of K, Ca, Na, Mg, P, Zn (Wu et al., 2017). In this study, microbial communities in the rhizosphere and bulk soil were sequenced in diseased and disease-free tomato plants to provide a theoretical basis for the dynamics of enzyme activities and microbial community in the rhizosphere and bulk soils of tomatoes influencing development of blossom-end rot disorder.

2. Materials and methods

2.1. Materials

Five cultivars of tomatoes (Mengyu, Xiuyu, Moyu, Meiyu and Sufen 11) were planted in natural soil in glasshouse with 1 m inter-row and 50 cm intra-row spacing after the application of 1500 kg per hectare of basal compound fertilizer (N-P-K = 15-15-15) at Xinyang Experimental Station of Jiangsu Academy of Agricultural Sciences (located in Dafeng, Yancheng, 32° 59′ N, 120° 50′ E). Five replicate plots (5 × 5 m each) were planted for every cultivar. The soil properties were: salt content 3.5–4.0 $g{\cdot}kg^{-1}$, pH 7.79, OC 53.42 $g{\cdot}kg^{-1}$, TN 6.15 $g{\cdot}kg^{-1}$, TP 4.72 $g{\cdot}kg^{-1}$, TK 36.63 $g{\cdot}kg^{-1}$, AP 27.48 $mg{\cdot}kg^{-1}$, AK 144.35 $mg{\cdot}kg^{-1}$. During growth, tomatoes were fertilized by top-dressing twice, each time with 75 kg per hectare of urea and 150 kg per hectare of the above-mentioned compound fertilizer.

Tomato plants with BER are not caused by human factors. Samples of tomato plants were collected on July 28 when most fruits were mature. We collected tomato roots, stems, leaves and fruits from randomly selected healthy plants as well as those with BER. The three replicate samples in each replicate plot were washed in running tap water and blotted with the absorbent paper and then placed in paper bags, respectively, and were oven-dried at 105 °C for 30 min to inactivate the enzymes, followed by drying at 75 °C until constant weight, grinding and storage in zip-lock bags (Wu et al., 2017; Shao et al., 2016).

The rhizosphere soil (collected by gently shaking the entire plant root system to remove loosely attached soil) and bulk soil samples (close to and away from tomato roots, respectively) were randomly collected at three points in each replicate plot from BER and healthy plants of each tomato cultivar. In the lab, rhizosphere and bulk soils were air-dried, crushed and sieved through a 0.15-mm sieve, mixed evenly and stored in zip-lock bags at room temperature for soil biological and chemical analysis (Chenery et al., 2012). Meanwhile, three replicates of the rhizosphere and bulk soils of each of the three tomato cultivars (Xiuyu, Moyu and Meiyu) were randomly selected and snap-frozen immediately in liquid nitrogen, and stored at −80 °C when returned to the laboratory, for subsequent DNA extraction.

3. Methods

3.1. Digestion of plant and soil samples

Plant samples (0.5 g, accurate to 0.0001 g) were microwave-digested in 5 mL of 0.5 M HNO_3 and diluted to 50 mL with ultra-pure water. Blank controls were run in parallel. Soil samples were digested according to the method used by Kingston and Jassie (1988).

3.2. Determination of soil pH and salt content

Preparation of soil extracts: 20 g of air-dried and sieved soil was mixed with 100 mL deionized water (1:5) in a 200-mL beaker and filtered through qualitative filter paper. Soluble salts in soil were determined by mass method using 50 mL of the soil water extract evaporated to dryness in a boiling water bath (Yang et al., 2016). A few drops of 30% v/v hydrogen peroxide were added to wet the residue; the procedure was repeated until the residue became white, followed by drying in an oven at 105–110 °C until constant weight. After cooling, weight was recorded. The quality of the evaporation dish is denoted as M1, the mass of the evaporating dish and dry soil sample is denoted as M2, while the quality of the soil weighed before extraction is denoted as M3.

The total amount of water-soluble salts in soil ($g\ kg^{-1}$) = (M2 − M1)/M3 × 2000where M1 – mass of evaporating dish (g); M2 – mass of evaporating dish and dry soil sample (g); M3 – the mass of soil before extraction (g).

Soil pH was determined by potentiometry after dry soil sample was mixed with carbon dioxide-free water at 1:2.5 ratio, with a calibrated pH meter (PHS-3C, Leici, Shanghai, China).

3.3. Catalase

Determination of hydrogen peroxidase activity (mL $KMnO_4{\cdot}kg^{-1}{\cdot}min^{-1}$) in soil samples was performed by using potassium permanganate titration method. Air-dried and sieved soil (5 g) was mixed with 40 mL of distilled water and 5 mL of 0.3% v/v H_2O_2, shaken by hand, followed by adding 5 mL of 1.5 mol L^{-1} sulfuric acid to stabilize the undecomposed hydrogen peroxide. The suspension was filtered (qualitative filter paper, pore size: 2.5 μm) and titrated to pale pink with 0.02 M potassium permanganate.

3.4. Invertase

Soil invertase activity ($mg{\cdot}kg^{-1}{\cdot}d^{-1}$) was determined by 3,5-dinitrosalicylic acid colorimetry. Ten grams soil was mixed with 30 mL of 8% (v/v) sucrose solution, 10 mL of phosphate buffer, and 1 mL of toluene, and was incubated at 37 °C for 24 h. After incubation, the samples were filtered through qualitative filter paper. An aliquot of 2–10 mL of the filtrate (20 mL for blanks without sucrose) was added to a 50 mL volumetric flask containing 3 mL of DiNitroSalicylic reagent, kept in a boiling water bath for 5 min, followed by placing flasks under running cool water for 3 min. The solution was made up to 50 mL with distilled water, and an aliquot of 200 μL was transferred to an ELISA plate; absorbance was read on a microplate reader at 508 nm. The standard curve was prepared using glucose. The invertase activity was expressed in milligrams of reducing sugar per gram of soil at 37 °C for 24 h.