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Effects of soil sterilization and biological agent inoculation on the root respiratory metabolism and plant growth of *Cerasus sachalinensis* Kom.

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ABSTRACT

Plant growth and development is affected by soil environment, which is rich in soil microbes. In this study, three treatments [biological agent inoculation (BA), soil sterilization (SS), and soil sterilization plus biological agent inoculation (SSBA)] were used to determine the effects of microorganisms on the respiratory metabolism and growth of *Cerasus sachalinensis* Kom. seedlings. Results showed that the microbial biomass carbon was significantly decreased, whereas the height of the seedlings was increased in the SS treatment. Glycolysis, tricarboxylic acid cycle, and cytochrome oxidase pathways were the dominant respiratory pathways from 5 d to 50 d after *C. sachalinensis* seedlings were subjected to SS, BA, and SSBA treatments. The variation trends of the tota lroot respiration rate, which increased initially and decreased subsequently, was the same in the three treatments. The total respiration rate of the roots in the three treatments was significantly lower than that in the control treatment at 20 d, whereas it was significantly higher than that in the control in SSBA treatment at 50 d only. Phosphofructokinase, malate dehydrogenase, and succinate dehydrogenase activities significantly changed in response to the three treatments, but pyruvate kinase activity significantly varied in SS and SSBA treatments. Therefore, SS and SSBA treatments were found to be beneficial for the growth of *C. sachalinensis* Kom. as determined by the significant increase in the height of the seedlings.

1. Introduction

Rhizosphere microorganisms have an important function in the soil-plant ecological system. Many researchers have focused on the improvement of crop growth and development by regulating the soil microbial environment, such as beneficial microorganism inoculation and soil sterilization. In particular, inoculation of arbuscular mycorrhizal fungi is known to alleviate soil compaction and promote wheat nutrient absorption (Miransari et al., 2009). Erturk et al. (2010) inoculated kiwifruit with growth-promoting bacteria, which had the ability to produce auxin (indole-3-acetic acid, IAA) and increase the rooting rate of kiwifruit cuttings. Gholami et al. (2009) showed that the growth-promoting bacteria could improve the germination percentage of maize seeds and enhance seedling vigour. The promoting effects of beneficial soil microorganisms, such as the production of IAA and enhancement of plant systemic stress tolerance, have been confirmed through several experiments (Ahmad et al., 2008; Yang et al., 2009). However, the community structure and soil microorganism activity differ under various soil environments and species (Øvreås and Torsvik, 1998). Hence, stable results similar to those obtained in laboratories are difficult to achieve. The response mechanism of physiological metabolisms, such as root respiration, to the changes in soil microbial environment needs further investigation.

Cerasus sachalinensis Kom. is native to the northeastern region of China and North Korea. It is widely used as a rootstock of sweet cherry in cool regions such as Dalian and Qinhuangdao because of several properties such as high propagation, cold resistance, and good graft compatibility with sweet cherry. In China, the planting area of sweet cherry measured 134,000 ha in 2011; one-fourth of the plants were cultivated using *C. sachalinensis* as rootstocks.

Abbreviations: BA, biological agent inoculation; SS, soil sterilization; SSBA, soil sterilization plus biological agent inoculation; IAA, indole-3-acetic acid; EMP, glycolysis; TCA, tricarboxylic acid cycle; PP, pentose phosphate; COX, cytochrome oxidase; AOX, alternative oxidase; HK, hexokinase; PFK, phosphofructokinase; PK, pyruvate kinase; MDH, malate dehydrogenase; IDH, isocitrate dehydrogenase; SDH, succinate dehydrogenase; G-6-PDH, glucose-6-phosphate dehydrogenase; 6-PGDH, 6-phosphate glucose dehydrogenase.

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However, long-term culture has revealed that the resistance of *C. sachalinensis* roots decreases rapidly when cherry plants reach the fruiting stage, leading to the reduction of tree vigour. Lü et al. (2011) reported that the respiratory function of roots significantly decreases in cherry orchards with heavy soil and poor soil permeability, which is always accompanied by changes in the microorganism community structure in the rhizosphere as well as reduction of tree vigour. There is also an increase in the number of pathogenic microorganisms in the rhizosphere, such as *Agrobacterium*.

Respiration is the fundamental energy-conserving process common to all living organisms; it leads to the generation of ATPs needed for cell maintenance and growth (Millar et al., 2011). Growth rate is a function of respiratory rate and efficiency (Hansen et al., 2002). Considering that plant growth is affected by soil microbes, there should be a relationship between soil microbes and plant respiratory metabolism. The relationships between soil microbial properties and root functions of *C. sachalinensis* were investigated by sterilizing the soil and inoculating with beneficial microbial agents. The changes in root respiration processes, plant growth, and response pattern to soil microbial environment were also assessed. The present study aimed to provide a theoretical basis for the scientific development of soil management strategies and improving the rhizospheric environment as well as root function.

2. Materials and methods

2.1. Materials and treatments

The experiment was conducted at the Shenyang Agricultural University (41°83′N, 123°56′E), and its elevation, averages of accumulated temperature, annual sunshine hours, annual frost-free period, and mean annual precipitation were 76.2 m, 3281 °C, 2372 h, 146–163 d, and 721 mm, respectively. The cultivation soil was constructed using garden soil, furnace ash, and organic fertilizer [3:1:1 (v:v:v)]. The basic physical and chemical properties of the soil were as follows: pH 7.35; soil organic matter, 42.5 g kg^{-1}; available nitrogen, 118.5 mg kg^{-1}; available phosphorus, 106.1 mg kg^{-1}; and potassium, 278.9 mg kg^{-1}. Portions of the soil were treated at 165 °C for 6 h. After sterilization, sterilized and non-sterilized soil were placed in plastic pots (diameter = 10 cm) and kept in a greenhouse for growth of the test seedlings. The seedlings were sowed in April 2011 and transplanted to pots when five to six leaves began to appear. Eight-leaf seedlings with a consistence growth potential were used as materials. The biological agent used was fermented using a mixture of *Trichoderma viride* TR-8 and *Bacillus* B67 strain, which was developed at the Biopesticide Engineering Center of Shenyang Agricultural University and the Institute of Biological Control of the U.S. Department of Agriculture. This mixture efficiently improves the soil environment, controls wilt, and promotes plant growth (Ji et al., 2002).

The soil treatments used were as follows: biological agent inoculation (BA), soil sterilization (SS), and soil sterilization plus biological agent inoculation (SSBA). Non-sterilized and non-inoculated soil was used as the control treatment.

The seedlings for BA and SSBA treatments were placed in non-sterilized and sterilized soil, respectively, and treated with the biological agent at a concentration of 10 g L^{-1} in June. The determined suspensions (10 mL per pot) were used to treat the soil every 5 d for 30 d, and the seedlings were treated for total six times. The control and SS treatments were supplied equivalent volume of tap water and sterilized water, respectively. The roots were sampled at 5, 20, 35, and 50 d after the last treatment (5 d, 20 d, 35 d, and 50 d refer to 5 d, 20 d, 35 d and 50 d after the seedlings were treated for the last time, respectively). The experiments were performed in three replications. The mass growth of the seedlings was determined at 5 d with 10 replications.

2.2. Experimental method

The microbial biomass carbon was detected by chloroform fumigation (Jenkinson and Powlson, 1976; Vance et al., 1987). Fresh soil samples (10 g dry weight) were fumigated for 24 h with alcohol-free $CHCl_3$ and extracted with 0.5 M K_2SO_4. A second non-fumigated set of soil samples was extracted during the 24-h fumigation without alcohol-free $CHCl_3$. The mixture of filtered soil extracts and 0.018 M $K_2Cr_2O_7$-12 M H_2SO_4 was digested at 175 °C and then the excess $K_2Cr_2O_7$ was titrated with $FeSO_4$. Microbial biomass carbon was calculated using the following equation: Microbial biomass carbon = E_c/0.38, where E_c = [12 × 10^3 × (volume of fumigated soil minus volume of non-fumigated soil) × concentration of $FeSO_4$ × dilution multiple]/weight of dry soil.

Root respiration rate was measured as oxygen consumption by using an Oxytherm oxygen electrode (Hansatech, England) according to a modification of Bouma's method (2001). The total respiratory rate was determined by slicing new root-tissue samples (width, 1.5 mm; length, 2–3 cm; 50 mg) into 2-mm pieces and setting them aside for 15 min to eliminate the influence of wound respiration. Subsequently, 0.05 g of each sample was weighed for measurement, and six replicates were incubated at 25 °C ± 1 °C. Total respiration rates were determined as root O_2 uptake per unit fresh mass (μmol min^{-1} g FM^{-1}) in the assay buffer without inhibitors.

Root respiratory pathways were measured according to Yu and Pan (1996). The capacities of the glycolysis (EMP), tricarboxylic acid cycle (TCA), and pentose phosphate (PP) pathways were determined using 0.5 M NaF, malonic acid, and Na_3PO_4 as inhibitors, respectively. The cytochrome oxidase (COX) and alternative oxidase (AOX) pathways were inhibited using 0.1 M NaCN and salicylhydroxamic acid, respectively. Phosphate buffer (0.2 M, pH 6.8) was used as the reaction medium. Each respiratory pathway was determined three times. The percentage of each respiratory pathway was calculated as [(total respiration rate – residual respiration rate)/total respiration rate]. The residual respiration rates represented respiration rates after the addition of the corresponding inhibitor.

Root tissues (0.5 g) were pulverised in 3 mL extraction buffer (100 mM Tris-HC1, pH 7.5) by using a mortar and pestle chilled on ice. The mixture was centrifuged for 30 min at 10,000 × *g* at 4 °C, and the supernatant was assayed immediately for 3 min in 3.0-mL reaction mixtures: hexokinase (HK, EC 2.7.1.1) 60 mM $MgCl_2$, 1 mM DTT, 0.5 mM $NADP^+$, 2.0 mM ATP, 15 mM glucose, 2 U mL^{-1} glucose 6-phosphate dehydrogenase (Harrison, 1971); phosphofructokinase (PFK, EC 2.7.1.11) 4 mM fructose-6-phosphate, 10 mM $MgSO_4$, 1 mM DTA, 4 mM ATP, 0.15 mM NADH, 2U aldolase, glucose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase (Harrison, 1971); pyruvate kinase (PK, EC 2.7.1.40) 1 mM DTT, 10 mM KCl, 15 mM $MgCl_2$, 0.5 mM PEP, 0.25 mM NADH, 5.0 mM ADP, 10 U mL^{-1} lactate dehydrogenase (Randall and Anderson, 1975); malate dehydrogenase (MDH, EC 1.1.1.37) 10 mM $MgCl_2$, 5 mM glutathione, 5 mM NADH, 10 mM oxaloacetic acid (Bryce et al., 1976); isocitrate dehydrogenase (IDH, EC 1.1.1.41) 20 mM $MgSO_4$, 10 mM $NADP^+$, 67 mM isocitrate (Collins and Merrett, 1975); glucose-6-phosphate dehydrogenase (G-6-PDH, EC 1.1.1.49) 6 mM $MgCl_2$, 1 mM DTT, 1 mM $NADP^+$, 2 mM glucose 6-phosphate (Lamed and Zeikus, 1980); and 6-phosphate glucose dehydrogenase (6-PGDH, EC 1.1.1.44) 6 mM $MgCl_2$, 1 mM DTT, 1 mM $NADP^+$, 2 mM 6-phosphogluconicacid trisodium salt (Lamed and Zeikus, 1980). All enzymatic assays were performed at 25 °C in 3 mL final volume. Oxidation of NADH or reduction of $NADP^+$ was measured