horticulturae

MDPI

Supplementary data

Effects of crystal lime sulfur fumigation and application of root growth promoting agents on the control of apple replant disease

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2.3.2. Chlorophyll content of Malus hupehensis Rehd. Seedlings

$C_{chlorophyll\ a}=13.95D_{665nm}-6.88D_{649nm}$

$C_{chlorophyll\ b}=24.96D_{649nm}-7.32D_{665nm}$

$C_{carotenoid}= (1000\ D_{470nm}-2.05C_{chlorophyll\ a}\ -114.8C_{chlorophyll\ b})\ /245$

Pigment content (mg/gFW) =C*V*n/W (V=0.02 L，n=1，W=0.2 g)

2.3.3. Determination of Root Respiration Rate and Root Protective Enzyme Activity

MDA: Take 1 mL of supernatant for each treatment, add 1 mL of clear water for the control treatment, and then add 2 mL of 0.67% TBA (accurately weigh 0.67 g of TBA reagent, add a small amount of 1 mol·L^{-1} NaOH solution, and finally use 10% trichloroacetic acid solution to fix the volume to 100 mL) into each treatment. After sealing, add it to boiling water, boil it for 15 min, and rinse the test tube with running water until rapid cooling. Then, pour it into a finger-shaped tube, and centrifuge it for 20 min at 4000 rpm. Finally, take the supernatant, and compare it with the spectrophotometer at 450 nm, 532 nm, and 600 nm.

SOD: Accurately weigh 0.5 g of the root sample, add 1 mL of phosphoric acid buffer (0.05 mol·L^{-1}, pH = 7.8) into the sample, and mix it evenly. Grind it fully under ice-bath conditions. Then, prepare 1 mL of phosphoric acid buffer, and add it again. Pour it all into the centrifuge tube, and then rinse the mortar with 2 mL of phosphoric acid buffer. Pour all the residual liquid into the centrifuge tube, balance the weight of both sides, and adjust the centrifuge condition to 10,500 rpm at low temperature (4 ℃) for 20 min. After centrifugation, store at – 20 ℃. Take exactly the same test tube, and add 50 μL of suspension. In the control treatment, add 50 μL phosphate -buffered solution, and then add 3 mL of reaction solution (water –phosphate buffer– Met– NBT– EDTA-Na_2: FD = 5:30:6:6:6). After that, treat a group of controls in the dark, and keep the other treatments in 4000lx sunlight for 30 min. After the reaction is over, take the dark-treated reference tube as a blank, perform colorimetry under the wavelength of 560 nm, and determine the absorbance values of different treatments.

CAT: Add a 100 μL volume of suspension into a plastic cup (add the control with phosphorus to slow down by 100 μL). Next, add 3 mL of reaction solution (20 mL, 0.1 mol·L^{-1}, pH = 7 phosphoric acid buffer, and finally, add 5 mL, 0.1 mol·L^{-1} H_2O_2), read the OD value of 240 nm using the kinetic curve of the spectrophotometer and time it. Read it every 1 min (read the OD value of 0, 1, 2, and 3 min), and read it 3 times continuously.

POD: Add a 20 μL volume of suspension into the plastic cup as a control variable, and add 20 μL of phosphoric acid buffer solution to the control treatment. Then, add 3 mL of reaction solution (50 mL, 0.1 mol·L^{-1}, pH = 6 phosphoric acid buffer solution, add 28 μL guaiacol), and finally, add 19 μL 30% H_2O_2. Use the kinetic curve of the spectrophotometer to read the OD value at 470 nm and time it. Read it every 1 min (read the OD value at 0, 1, 2, and 3 min), and read it 3 times continuously.

2.3.4. Determination of Soil Enzyme Activity

Soil urease activity was determined by a colorimetric assay using sodium phenate–sodium hypochlorite. First, 5 g of air-dried soil samples was weighed in a 50 mL triangulated flask, and 1 mL of toluene was added, followed by shaking until the contents were evenly mixed. After 15 min, 10 mL of 10% urea solution and citrate buffer solution was added, followed by shaking and incubation at 37℃ for 24 h. After culture, the filtrate was filtered, and 1 mL of filtrate was added into a 50 mL volumetric flask. Next, 4 mL of sodium phenol solution and 3 mL of sodium hypochlorite solution were added and shaken well. After 20 min, the mixture was diluted to the 50 mL mark, and the spectrophotometer was colorimetric at 578 nm (the blue of indophenol remained stable). Urease activity was calculated by subtracting the absorbance value of the sample from the difference in the absorbance value of the control sample, and the ammonia nitrogen content was calculated according to the standard curve.

The activity of urease (Ure) was represented by the ammonia-nitrogen content (mg) in 1 g of soil after 24 h. The formula for determining soil urease activity was as follows:

$$Ure=a*V*n/m \quad (1)$$

where a is the concentration of ammonium-nitrogen obtained from the standard curve (mg·mL^{-1}), V is the volume of the chromatic liquid (50 mL), n is the separation multiple, and m is the weight of the drying soil (g).

Soil phosphatase activity was determined by a colorimetric assay with disodium phenyl phosphate. First, 5 g of air-dried soil samples was placed in a 200 -mL triangulation flask, and 2.5 mL of toluene was added. After shaking for 15 min, 20 mL of 0.5% benzene-disodium phosphate was added. After shaking, the samples were placed in an incubator and cultured at 37℃ for 24 h. Next, 100 mL of 0.3% aluminum sulfate solution was added to the culture medium and filtered. After that, 3 mL of filtrate was then absorbed into 50 mL volumetric bottles, and 5 mL of buffer solution and 4 drops of chlorodibromo-p-benzoquinone imines reagent were added to each bottle. After color development, the solution was diluted to the scale, and the colorimetric determination was conducted 30 min later. The boric acid buffer was blue and colorimetric at 660 nm on the spectrophotometer. To draw the standard curves, 1, 3, 5, 7, 9, 11, and 13 mL of phenolic working fluids were taken for color development and volume determination. After color stability was achieved, the standard curve was drawn with the colorimetric method. Phosphatase activity was expressed in phenolic micrograms per gram of soil.

The activity of phosphatase (Pho) was represented by the phenol content (mg) in 1 g of soil after 24 h. The formula for determining soil phosphatase activity was as follows:

$$Pho=a*V*n/m \quad (2)$$

where a is the concentration of phenol obtained from the standard curve (mg·mL^{-1}), V is the volume of the chromatic liquid (50 mL), n is the separation multiple, and m is the weight of the drying soil (g).

Soil invertase activity was determined by the colorimetric assay with 3,5-dinitrosalicylic acid. First, 5 g of air-dried soil samples as placed in a 50 mL triangulated flask, and 10 mL of 1% starch solution was injected. This was followed by the addition of 10 mL of pH 5.6 phosphate buffer solution and 5 drops of toluene with shaking and storage in an incubator. The samples were then cultured at 37℃ for 24 h. After culture, the suspension was filtered. Next, 1 mL of filtrate was poured into a 50 mL volumetric flask. Then, 2 mL of 3, 5-dinitrosalicylic acid solution was added and heated in a boiling water bath for 5 min. The solution was then moved to the volumetric flask to the running water to cool.

After a constant volume of 50 mL was achieved, colorimetry was performed at 508 nm on a spectrophotometer. Glucose solution was used as the standard.

The activity of invertase (Inv) was represented by the glucose content (mg) in 1 g of soil after 24 h. The formula for determining soil invertase activity was as follows:

$$Inv=a*V*n/m \quad (3)$$

where a is the concentration of glucose obtained from the standard curve ($mg \cdot mL^{-1}$), V is the volume of the chromatic liquid (50 mL), n is the separation multiple, and m is the weight of the drying soil (g).

Soil catalase activity was determined by potassium permanganate titration. First, 2 g of air-dried soil samples was placed in a 100 mL trigonometric bottle and injected with 40 mL of distilled water and 5 mL of 0.3% hydrogen peroxide solution. A control (40 mL of distilled water and 5 mL of 0.3% hydrogen peroxide solution) was injected into a triangular bottle without the addition of soil samples. The triangular bottle was shaken on a shaking machine for 20 min, and 5 mL of 3N sulfuric acid was added to stabilize the undecomposed hydrogen peroxide. The suspension in the bottle was then filtered with a slow filter paper, followed by absorption of 25 mL of filtrate and titration with 0.1 N potassium permanganate to the light pink terminal point.

The activity of catalase (Cat) was represented by volume (mL) of 0.1 N potassium permanganate in 1 g of soil after 20 min. The formula for determining soil catalase activity was as follows:

$$Cat=(A-B) * T \quad (4)$$

where B is the amount of potassium permanganate (mL) consumed for titrating the soil filtrate (mL), A is the amount of potassium permanganate (mL) consumed for titrating 25 mL of the original hydrogen peroxide mixture (mL), and T is the correction value for potassium permanganate titration.

The processing and analysis of the amplicon sequencing

The bacteria and fungi PCR products were purified and pooled in equimolar amounts and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw fastq files of bacterial and fungal reads obtained from MiSeq sequencing were quality-filtered by Trimmomatic and merged by FLASH (version 1.2.11) (Magoc and Salzberg, 2011; Bolger et al., 2014; Gdanetz et al., 2017). The processed sequences were subsequently clustered into operational taxonomic units (OTUs) with a minimum of 97% similarity using UPARSE (version 7.0.1090) (Gdanetz et al., 2017). The taxonomy of the bacterial sequence was analyzed by the RDP Classifier algorithm against the SILVA database (version 138/16S-bacteria database) with a confidence threshold of 70%. The taxonomy of the fungal sequence was analyzed by the RDP Classifier algorithm against the United States database (version 8.0/ its-fungi database) using a confidence threshold of 70%. Potential functional changes of the bacterial community in the soil samples were predicted using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2.2.0) (Huang, 2019).

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