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Penicillium–sesame interactions: A remedy for mitigating high salinity stress effects on primary and defense metabolites in plants

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ABSTRACT

Salinization of agricultural land is increasing, and reduces the yield of crop plants. The introduction of plant growth-promoting and salt-tolerant microbes to soil can protect plants from salt effects. This study examined the primary and defense metabolites in sesame plants associated with *Penicillium* sp. NICS01, which mitigates oxidative stress induced by high salinity. Salt tolerance in *Penicillium* sp. NICS01 was observed in medium supplemented with 5.0% and 7.5% NaCl. The role of this fungus in mitigating against high salinity (150 mM NaCl) was tested on salt-stressed sesame plants. Salt stress decreased the length and weight of sesame shoots, but applying *Penicillium* sp. NICS01 significantly ($p \leq 0.05$) increased these parameters in plants grown under salt-stress conditions by enhancing photosynthetic pigment levels (chlorophylls and carotenoids), sugar concentrations (sucrose, glucose, and fructose), fatty acid contents (palmitic acid, linolenic acid, arachidic acid, and *cis*-11-eicosenoic acid), and ionic transport (K and Ca, ($p \leq 0.05$)). In addition, salt-induced oxidative damage was reduced by lowered lipid peroxidation (5%) and salicylic acid (15%) and Na (18%) contents, and raised peroxidase activity (more than five-fold), while amino acid (Thr, Gly, Val, Met, Ile, Leu, Tyr, Phe, Arg, Asp, Ser, Asn, Glu, Ala, and GABA) synthesis was regulated by the fungal interaction. Asp, Thr, Ser, Glu, Ala, and Arg contents were significantly ($p \leq 0.05$) enhanced in salinity affected plants due to the effect of fungal inoculation. Our findings revealed that *Penicillium* sp. NICS01 regulates the biosynthesis of primary and defense metabolites in sesame plants under salt stress, suggesting that this fungus can ameliorate damage caused by salt stress in crop plants.

1. Introduction

Sesame (*Sesamum indicum*, Pedaliaceae) is widely grown under various environmental conditions in tropical and subtropical countries. The nutritional benefits of sesame seeds, such as high oil concentration and protein content, have promoted research to enhance productivity. In particular, sesame oil is more stable than that of other crops because the double bonds in the unsaturated fatty acids sesamin and sesamolin are strong (Anilkumar et al., 2010; Uzun et al., 2002). The growth and yield of sesame is limited by low genetic yield potential and by biotic and abiotic factors (Jyothi et al., 2011; Radhakrishnan et al., 2014). Salinization of agricultural land is an important constraint on plant growth. Low rainfall, intensive use of irrigation, and applied fertilizers are major causes to soil salinity. Sodium chloride (NaCl) is the most abundant salt in saline environments (Tester and Davenport, 2003). The high concentrations of toxic Na^+ and Cl^- ions in saline soils are taken up by roots and transported to shoots and leaves through xylem, where their excessive accumulation inhibits the uptake of other ions. This ionic imbalance causes oxidative stress via the production of reactive oxygen species (ROS), which inhibit many physiological and biochemical processes in plants and alter the metabolism of carbohydrates, amino acids, and fatty acids (Verma and Mishra, 2005).

Nitrogen fixation by symbiotic microbes has been widely reported. Such microorganisms associated with plants support plant growth via their abilities to produce phytohormones, solubilize insoluble phosphate, and convert complex organic substances to simple forms. Under salt stress, fungal symbionts regulate ionic transport in plants and reprogram physiological changes induced by oxidative stress. The activation of antioxidants such as catalase, peroxidase, and superoxide dismutase in fungal associates of plants during salt stress reduces lipid peroxidation and ROS, preventing salt-induced damage (Radhakrishnan et al., 2013a). Recently, we reported that exogenous treatment with fungi is an environmentally friendly way to improve the growth of sesame plants by preventing disease and salt stress (Radhakrishnan et al., 2013b,c, 2014). In our earlier studies, we identified

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Penicillium sp. NICS01 as a potent soil fungus that benefits sesame growth under both *Fusarium* infection and non-infection conditions (Radhakrishnan et al., 2013b). This study was conducted to assess biochemically the ameliorative effects of *Penicillium* sp. NICS01 on sesame under salt stress.

Very few reports have documented how fungi affect amino acid, fatty acid, and sugar metabolism in salt-stressed plants. Plants tolerate saline conditions via multiple biochemical pathways that synthesize osmotically-active metabolites, protect chloroplast function, and maintain water and ion homeostasis (Parvaiz and Satyawati, 2008). Sesame can tolerate moderate soil salinity (Yousif et al., 1972), but detrimental effects of salt stress have been documented by several authors (Koca et al., 2007; Yahya 2010; Bazrafshan and Ehsanzadeh, 2014). Recently, *Penicillium* sp. induced amelioration of salt stress effects in crop plants has been reported (Khan et al., 2011; Radhakrishnan et al., 2014). To date, there are no reports on *Penicillium*-induced changes in amino acids, fatty acids, and sugars, and salicylic acid (SA) in sesame under such conditions, therefore, we hypothesized that their interaction might help to avoid the oxidative stress due to the regulation of those principal growth responsible metabolic contents in host-plants infected at soil salinity. This study investigated the mechanisms by which *Penicillium* sp. NICS01 interact with sesame plants under high salinity by analyzing primary and defense metabolites including photosynthetic pigments, sugars, fatty acids, amino acids, ionic transport, lipid peroxidation, antioxidants, and stress hormone.

2. Materials and methods

2.1. Penicillium sp. NICS01 growth under salinity

Penicillium sp. NICS01, which produces indole acetic acid and amino acids, was isolated from rhizosphere soil of peanut plants grown at the National Institute of Crop Science, Miryang, Republic of Korea, and cultured on potato dextrose broth (PDB). NaCl at the concentrations of 2.5%, 5.0%, 7.5%, and 10.0% was added to the PDB medium for salinity treatments. *Penicillium* sp. NICS01 was inoculated into autoclaved saline and non-saline media. Fungal growth colonies were measured at 10 h, 15 h, 25 h, 35 h, 13 d, and 17 d by counting colonies. To know the health and survivability of the fungus under salt stress condition, it was allowed to grow on PDA medium, and fungal colony morphology and pigmentation were observed.

2.2. Fungal inoculation and salinity stress on sesame plants

Sesame (*Sesamum indicum* L. cv. Jinju) seeds were obtained from the sesame germplasm collection of the National Institute of Crop Science. Seeds were soaked in running tap water and surface-sterilized by adding 0.5% sodium hypochloride, followed by thorough rinses with sterile distilled water. An artificial soil mixture (13–18% (w/v) peat moss, 7–11% perlite, 63–68% coco-peat, and 6–8% zeolite, ~90 mg/kg NH^{4+}, ~205 mg/kg NO_{3-}, ~350 mg/kg P_2O_5, and ~100 mg/kg K_2O) was autoclaved and used to fill a seedling plug tray (50 cells) that had been sprayed with ethanol and washed with sterile water. The sterilized seeds were planted in the seedling tray, and uniformly-sized 3-week-old-seedlings were transferred to pots (30 × 15 cm) containing artificial soil mixture. Sterile water was added at regular intervals. Ten milliliters of *Penicillium* sp. NICS01 (2.1×10^4 CFU/mL) were applied to soil containing 4-week-old-sesame plants. After 2 weeks of fungal treatment, 150 mM NaCl was added to the soil. Plant growth parameters, i.e., shoot length and biomass, under salinity and fungal treatments were analyzed at the wilting stage 17 d after salt stress. The plants were harvested and stored at −80 °C, and freeze-dried samples were used to analyze primary and defense metabolites. To determine the fungal association with sesame plants, 17-d-old plant roots infected with fungus were cleaned and treated with sodium hypochlorite (3%) for 10 min, and rinsed with sterilized distilled water. The roots were sectioned and assessed through light microscope (Olympus BX50, Digital camera DP26) to observe the fungal colonies.

2.3. Primary metabolite analysis

2.3.1. Carbohydrates

The freeze-dried sesame samples were ground, and 1 g of sample was used for carbohydrate analysis. Sugar was extracted by adding 70% ethanol (Hinesley et al., 1992) and shaking at 240 rpm for 3 h. The supernatant was filtered through a Nylon-66 Syringe Filter (0.45 μm). The extracted sugar samples (20 μL) were injected to an HPLC machine with a sugar-pak column (300 mm) with Shodex R1-101 detector. Ca-EDTA in deionized water was used as mobile phase at a flow rate of 0.5 mL/min at 80 °C. The stachyose, raffinose, sucrose, glucose, galactose, and fructose contents were quantified based on peak areas and comparison with standard curves obtained using known standards.

2.3.2. Amino acids

Freeze-dried and ground sesame plant samples (50 mg) were dissolved in double-distilled water and centrifuged at 10,000 × *g* at 4 °C for 30 min. Amino acid were detected and quantified according to the method described by Radhakrishnan et al. (2013c). The supernatant was filtered through a nylon syringe filter (0.45 μm), and 0.1 mL was then mixed with 0.9 mL lithium citrate-loading buffer (pH 2.2). Amino acids and GABA were determined using a Biochrom 30 amino acid analyzer with a single lithium cation exchange resin column (4.6 × 200 mm), with ninhydrin as the color reactant. Chromatography data were analyzed using Ezchrom E software. The concentrations of individual amino acids (Asp, Thr, Ser, Asn, Glu, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, and Arg) and GABA were calculated by comparison with known concentrations in a standard solution containing the amino acids.

2.3.3. Fatty acids

Fatty acid contents in sesame plants were analyzed as described by Radhakrishnan et al. (2013c). Total lipids were extracted from plant samples by the Soxhlet method using a Buchi B-811 extraction system (Buchi Labotechnik, AG, Flawil, Switzerland). Fatty acid methyl esters (FAMEs) were prepared from total lipids by acid-catalyzed *trans*-esterification. One milligram of lipids was resuspended in toluene, and then methanolic H_2SO_4 (1%) was added. The reaction mixture was heated at 100 °C for 1 h, and 3 mL H_2O with 2 mL hexane was used to extract FAMEs. Fatty acids were analyzed as FAMEs using a capillary gas chromatograph equipped with a HP-FFAP capillary column (30 m × 0.318 mm, I.D. 25 μm film) and programmed to hold at 150 °C for 1 min, increase to 230 °C for 1 min, then to 240 °C for 2 min. Both the injector and detector were held at 260 °C. Nitrogen was used as carrier gas at 1 mL/min, the injection volume was 1 μL, and the split ratio was 1:50. The percentage of fatty acids was calculated from standard values of peak areas of palmitic, stearic, oleic, linoleic, linolenic, arachidic, *cis*-11-eicosenoic, and behenic acid methyl esters.

2.3.4. Photosynthetic pigments

Leaves of sesame plants were ground in 80% acetone to analyze photosynthetic pigments. Total chlorophyll and carotenoid contents were estimated according to the methods of Arnon (1949) and Lichtenthaler (1987), respectively. Pigments in the supernatant were detected at 663, 645, and 470 nm and their concentrations calculated using the following formulae: