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Research article

Salt tolerance of *Glycine max*.L induced by endophytic fungus *Aspergillus flavus* CSH1, via regulating its endogenous hormones and antioxidative system

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ABSTRACT

Abiotic stress resistance strategies are powerful approaches to sustainable agriculture because they reduce chemical input and enhance plant productivity. In current study, an endophytic fungus, *Aspergillus flavus* CHS1 was isolated from *Chenopodium album* Roots. CHS1 was initially screened for growth promoting activities like siderphore, phosphate solubilization, and the production of indole acetic acid and gibberellins and were further assayed for its ability to promote the growth of mutant *Waito*-C rice. The results revealed that different plant growth characteristic such as chlorophyll content, root-shoot length, and biomass production were significantly promoted during CHS1 treatment. This growth promotion action was due to the presence of various types of GAs and IAA in the endophyte culture filtrate. Significant up regulation with respect to levels in the control was observed in all endogenous plant GAs, after treatment with CHS1. Furthermore, to evaluate the potential of CHS1 against NaCl stress up to 400 mM, it was tested for its ability to improve soybean plant growth under NaCl stress. In endophyte-soybean interaction, CHS1 association significantly increased plant growth and attenuated the NaCl stress by down regulating ABA and JA synthesis. Similarly, it significantly elevated antioxidant activities of enzymes catalase, polyphenoloxidase, superoxide dismutase and peroxidase as compared to non-inoculated salt stress plants. Thus, CHS1 ameliorated the adverse effect of high NaCl stress and rescued soybean plant growth by regulating the endogenous plant hormones and antioxidative system. We conclude that CHS1 isolate could be exploited to increase salt resistant and yield in crop plants.

1. Introduction

Despite worldwide efforts over the last few decades to increase agricultural production to meet the needs of a rapidly growing population, the goal of reducing the problems associated with hunger has not yet been achieved (Waller et al., 2005). Plants are often exposed to adverse environmental conditions, and these abiotic stresses may play a key role in crop losses globally (Bybordi, 2012). One of the major abiotic stresses is salinity, which occurs worldwide in irrigated and non-irrigated regions. A wealth of literature is available on the detrimental effects of salinity on plant growth and production, particularly on crop plants (de Oliveira et al., 2013). Plants have evolved diverse mechanisms in response to the toxic effects of NaCl and low water potential in soil as a result of drought and salt stress (Munns and Tester, 2008). Generally, salt sensitive crops cannot endure elevated NaCl concentration, particularly in soil (Prasad et al., 2000), which causes reduced germination, growth, and biomass allocation (Ahmad and Prasad, 2011; Neumann, 2008). Many studies have shown that plants are more susceptible to salt stress during the vegetative and reproductive periods (Läuchli and Grattan, 2007; Siddiqui et al., 2014). Continued exposure of plants to salt stress leads to toxicity of specific ions, nutrients, and hormonal imbalance, and decreased water potential (Ahmad et al., 2010b; Siddiqui and Khan, 2013). Other influences of salt stress on plants include retarded enzyme activities, reduced

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effectiveness of carbon-use, poor germination, hyperionic and hyperosmotic effects, inhibited photosynthesis, and breakdown of protein and membrane structures.

Mutualistic symbiosis with endophytic and mycorrhizal fungi can enhance salt tolerance in plants and reduce yield losses in crops cultivated in saline habitats. *Piriformospora indica* is a member of the basidiomycete and a root endophyte that enhances the resistance of plants to root and leaf diseases and also mitigates salt stress in barley (Waller et al., 2005). Fungal endophytes belong to the ascomycetes or basidiomycetes, and live inside the roots or tissues of host plants without causing any harm (Arnold et al., 2007; Oses et al., 2008). Fungal endophytes enhance plant vigor by resisting herbivory and phytopathogens, and support plant growth by increasing nutrient uptake, water use efficacy, and mitigating environmental stresses (Richardson et al., 2009; Waller et al., 2005). In return, the endophytic fungi access nutrition via the host plant and propagate to subsequent generations, for example, members of Clavicipitaceous and Dikarya (Arnold et al., 2007; Iqbal and Ashraf, 2013). A wide range of bioactive metabolites are secreted by fungal endophytes. Fungal endophytes synthesize various plant hormones, such as gibberellins (GAs) (Khan et al., 2011b). Approximately 136 GAs have been identified to date in various infectious and nonpathogenic fungi associated with plants (Ahmad et al., 2010a). Several strains of fungi can produce compounds, such as IAA (indole-3-acetic acid) and abscisic acid (ABA). The IAA-producing strains include *Colletotrichum* sp. (Lu et al., 2000), *Talaromyces verruculosus* (Bhagobaty and Joshi, 2009), *Penicillium glabrum* (Hammerschmidt et al., 2012), *Williopsis saturnus*(Nassar et al., 2005), *Pi. indica* (Sirrenberg et al., 2007). The GAs-producing endophytic fungi include *Aspergillus fumigatus*, *Cladosporium sphaerospermum*, and *Turbo. funiculosus* (Khan et al., 2011c).

Soybean (*Glycine max*) growth is affected by different ecological factors (Radhakrishnan and Kumari, 2013). Soybean seeds are good sources of oil, protein, and isoflavonoids; however, the concentration of these is reduced in plants exposed to salt stress. Recently, eco-friendly techniques were proposed to alleviate the harmful influence of salt stress on soybean crop plants. To achieve this, it is necessary to identify and apply microbes that can promote soybean growth in saline soil. In this study, we isolated, identified, and characterized endophytic fungi from *Chenopodium album.* Furthermore, we hypothesized that such endophytic fungi synthesize various plant hormones that enhance plant growth under normal or unfavorable environmental conditions. Therefore, this study also evaluated the ameliorative effects of fungal endophytes on *G. max* against salinity based on antioxidants and phytohormones.

2. Material and methods

2.1. Isolation of endophytic fungi

Healthy *C. album* plants were collected from fields in Mardan (34° 12′ 22.0428″ N and 72° 1′ 47.2800″ E), Pakistan. Roots samples were carefully separated and kept in zip-lock bags for transfer to the laboratory and immediate processing. To remove soil particles and dust, stem tissues were washed using tap water and washed three times with distilled water. Stem tissues were cut into small segments of about 1 cm under hygienic conditions. Surface sterilization was conducted using 2.5% NaOCl for 30 min and 70% ethanol for 1 min. Then, the sterilized leaves were rinsed three times with double-distilled water. The final rinsing water was spread onto potato dextrose agar (PDA) plates to check the effectiveness of the sterilization process. The segments were placed on PDA plates provided with streptomycin (10 μg/mL) and incubated at 25 °C for 7 days. Fungal endophyte hyphae that grew from these samples were transferred to new PDA plates using Pasteur pipettes to obtain pure cultures. Pure culture samples were preserved in 15% glycerol at −70 °C for further analysis.

2.2. Screening endophytic fungi for IAA production

To assess the phytohormone production capacity of endophytes, an initial screening was conducted by adding 1 mL of Salkowski reagent to 2 mL of culture filtrate. Fungal isolate CHS1 was initially screened for IAA production using the colorimetric method (Ullah et al., 2013). The isolate was grown on Czapek-Dox broth medium in a shaking incubator at 120 rpm at 30 °C. After 7 days, the samples were filtered and the IAA concentrations of the culture filtrates were determined by adding 1 mL of Salkowski reagent to 2 mL of each culture filtrate, followed by incubation for 30 min in the dark. Strains positive for IAA production were selected for further study. One of the 12 initial endophytic isolates of *C. album* was found to produce IAA (the change in color from yellowish to pinkish indicated the presence of IAA). Isolate CHS1 was selected for further study based on IAA production and a morphologically distinct fungal strain.

2.3. Screening of isolate CHS1 on GA-deficient Waito-C dwarf rice

Dwarf phenotype *Waito-C* rice, with suppressed GA biosynthesis, was used to examine Culture filtrate (CF) of the isolate CHS1 for GAs. First, seeds were surface sterilized using 2.5% NaOCL for 30 min, then washed three times with distilled water and incubated for 24 h with 20 mg/L of uniconazole. Seeds were treated with uniconazole to block GA biosynthesis, and to check the effects of isolate CHS1 on *Waito-C* rice. The *Waito-C* seeds were pre-germinated and transferred to autoclaved pots containing horticultural soil with the following nutrient composition: peat moss (10–15%); perlite (35–40%); coco peat (45–50%); zeolite (6–8%); ~0.09 mg/g; ~0.205 mg/g ~0.35 mg/g P_2O_5; and ~0.1 mg/g K_2O to provide a microbe-free environment (Asaf et al., 2017; Khan et al., 2011b). When the seedlings were at the two-leaf stage, each seedling root was treated with 10 mL of a spore suspension of isolate CHS1, containing approximately 1000 spores. Only 10 mL of distilled water was added to control plants. After 22 days, chlorophyll content (SPAD-502; Minolta, Tokyo, Japan), root and shoot length, root and shoot fresh weight, and dry biomass were measured. Dry weights were measured after drying the plants at 70 °C for 48 h in an oven. Subsequently, all plants were harvested and stored in liquid nitrogen for further phytohormonal analysis.

2.4. Determination of phosphate solubilization and siderophore production

Fungal isolate CHS1 was inoculated into Pikovskayas (PVK) agar medium. Formation of a clear zone around the colony indicated phosphate solubilizing ability of the fungus (Premono et al., 1996). The strain was also assayed for siderophore activity using CAS agar medium (Vellore, 2001). Fungal strains that produced an orange halo around the fungal growth were considered as able to produce siderophores.

2.5. Determination of IAA content using GC/MS

The CF of isolate CHS1 was analyzed using GC/MS to assess IAA content. For this experiment isolate CHS1 was cultured in 50 mL Czapek-Dox broth provided with 0.5 mg/mL L-tryptophan and incubated in a shaking incubator at 200 rpm for 7 days at 28 °C. Cultures were filtered to obtain the culture filtrate. The exact determination of IAA in culture filtrates was quantified using SIM, 6890N network GC system, and 5973 network mass selective detector (Agilent Technologies, Santa Clara, CA, USA) according to the method described by Kang et al. (2015b). Finally, the methylated samples were again dissolved in ethyl acetate before being analyzed using GC/MS with selected ion monitoring capabilities (SIM; 6890N network GC system, and 5973 network mass selective detector; Agilent Technologies, Santa Clara, CA, USA). Results were calculated in μM per 50 mL, and the analysis was repeated twice. This experiment was performed three times.