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Original article

Gibberellin-producing *Serratia nematodiphila* PEJ1011 ameliorates low temperature stress in *Capsicum annuum* L.

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ABSTRACT

We studied the effects of plant growth-promoting rhizobacteria (PGPR) on the physio-hormonal attributes of pepper (*Capsicum annuum* L.) plants grown under low-temperature stress. After initial screening for growth promoting effect on gibberellin (GA) mutant *Waito-C* rice seeds, the PGPRs were analysed for gibberellins (GA) production through advanced chromatographic and spectroscopic techniques. Among 17 bacterial isolates, a novel isolate PEJ1011 produced bioactive GA_4 (8.65 ng ml^{-1}) and physiologically inactive GA_{20} (6.21 ng ml^{-1}) and GA_9 (1.64 ng ml^{-1}). The isolate PEJ1011 was identified as *Serratia nematodiphila* PEJ1011 using molecular techniques. To further assess it growth promoting effects, *S. nematodiphila* PEJ1011 was inoculated to pepper plant, where it significantly improved the growth attributes of pepper plants, while mitigated the deleterious effects of low temperature on pepper exposed to low temperature stress of 5 °C. It was observed that the inoculated plants grown under normal and low temperature stress contained higher endogenous GA_4 contents. To modulate cold stress, the beneficial association of PGPR up-regulated the endogenous ABA levels in pepper plants, while reduced the endogenous jasmonic acid and salicylic acid contents. This up and down regulation of stress hormones contribute to the immediate adaptation of plants exposed to low temperature stress. Current study showed the significance of *S. nematodiphila* PEJ1011 association to crops grown under adverse climatic conditions, and also reports the GA producing capacity of genus *Serratia* for the first time.

1. Introduction

Low temperatures greatly affect the physio-hormonal attributes of crops and reduce the crop productivity. The adverse effects of cold exposure include the hindrance of normal crop production, and in the worst situation, complete crop failure [20]. Crops may face stresses of varied origin, once or multiple times in their life cycle. However, sometimes a single, mild and brief stress is enough to eliminate the grower's profit. In the current global environmental scenario, it is predicted that the various stresses will increase in frequency and intensity [31]. Among the environmental stresses, low temperature stress is known to affect the vegetative and reproductive phases of the plant life cycle. Cold stress can cause flower abortion during reproductive phase due to abscission, sterility of both male and female organs, and eventually reduce yields due to unsuccessful fruit set, which affect all of humanity [75] [85]. Therefore, it is very important to adopt eco-friendly strategies, which can protect crop plants and enhance their tolerance to low temperature stress.

Phytohormones are key plant growth regulators and tend to facilitate physiological processes under normal and stressed conditions. The regulation of major phytohormones such as

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gibberellins (GAs), abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA) bear positive or negative effects on plant growth during cold stress [56]. GAs are considered to be plant growth promoters, while ABA, JA, and SA are grouped as stress-related hormones [48]. Phytohormones are known to interact with each other either in synergistic or antagonistic way, to modulate a particular physiological process under specific conditions and specific time [62]. During cold stress, each phytohormone has a distinguishable regulatory mechanism. ABA and SA are up-regulated, as the endogenous levels of both increases in response to low temperature stress. Treatment with ABA and SA increases the cold stress tolerance or acclimation to low temperatures in various crops such as winter wheat, spinach, potato tubers, maize, and rice [35,56]. During cold temperatures, GAs act antagonistically and their endogenous levels, along with that of auxin are down-regulated, causing dwarfism [1,56]. JA decreases the negative effect of chilling stress and improves the postharvest storage of fruits [68,80].

Plant growth-promoting rhizobacteria (PGPRs) are best known for their beneficial effects and amelioration of abiotic stresses in crops. The PGPRs actively colonize the rhizosphere and enhance the yield and resistance of host plants [2,77]. Plant growth promotion by PGPR includes solubilisation, mobilisation, and enhanced uptake of unavailable major plant nutrients, commonly known as biofertilisation and the modulation of phytohormones, or phytostimulation. The suppression of plant pathogens with the help of PGPRs is called biocontrol, and this mechanism involve indirect plant growth promotion [2,52,77]. The phytohormones production capacity of putative PGPRs have been reported for several bacterial spp. like *Bacillus cereus*, *B. macroides*, *B. pumilus*, *Azospirillum brasilense*, *Acinetobacter calcoaceticus* and *Burkholderia* sp. and these PGPRs were extensively studied for their role in plant growth promotion [21,41,42,45]. The phytostimulatory mechanisms include production of GAs, cytokinin, ABA, indole-3-acetic acid (IAA), ethylene, and JA by the PGPRs. The PGPR hormones supplement those produced by the plants and as a result the biomass and yield of the host plant increases [60,77], due to an increase in size, branches and large surface areas of the roots and leaves. An increased in surface area of the leaves allow maximum sunlight absorption and the long and highly branched roots facilitate higher nutrients uptake.

GAs are plant growth promoting hormones, which manipulate plant morphology and physiology by enhancing germination, promotes plant height, increase the number of flowers, promote floral organ development, quicken the onset of flowering and increase the leaf surface area [23,36,73,77]. However, there are few reports on PGPRs capable of GA production. Current study hypothesized that PGPR producing bioactive GAs might rescue plant growth under low temperatures by regulating endogenous GAs and related phytohormones, as reduced GAs biosynthesis is one of the major cause of dwarfism and malfunctioning of female sexual organs in crops during periods of low temperatures [69]. Therefore, we analysed the effects of a GA-producing PGPR (*S. nematodiphila* PEJ1011) on pepper under low temperature regime.

2. Materials and methods

2.1. Isolation of PGPR from pepper

The rhizospheric soil samples were obtained from randomly collected pepper plants roots grown in horticulture fields at Kyungpook National University, Republic of Korea as described in Refs. [8] and [34]. In order to remove the bulk soil, pepper plants were vigorously shaken by hand for 7 min, paying attention to the roots integrity. The actual limit for shaking was considered as reached when roots loose or non-adhering soil particles were completely removed [8]. The tightly adhered (rhizosphere) soil was then separated by using glass beads and then sieved with a 4 mm mesh [34].The rhizospheric soil samples were pooled together and about 10 g of rhizospheric soil was transferred to 250 ml flasks containing 100 ml of sterile Amies solution. The resulting suspensions were serially diluted (10^{-4}) and 0.1 ml aliquots were grown on tryptic soy/agar (TSA; Merck Co., Germany) for the isolation of bacteria. The isolated bacterial strains were repeatedly inoculated on new petri plates till purification was confirmed and then incubated for 48 h at 30 °C. Pure bacterial cultures were inoculated in nutrient broth with conditions as mentioned below and then screened for their plant growth promoting capacity by performing bioassay on dwarf gibberellins inefficient biosynthesis mutant *Waito-C* rice. The culture filtrate was harvested by centrifugation of culture broth at 5000 × *g* at 4 °C for 15 min. Supernatants (50 ml) were lyophilized in freeze dryer for 4 days, and then diluted in 1 ml of autoclaved distilled water. *Waito-C* seeds were surface sterilized with 2.5% sodium hypochlorite for 30 min, rinsed with autoclaved DDW, and then incubated for 24 h with 6.9 μM-uniconazol [38] to obtain equally germinated seeds. The equal size germinating rice seedlings were then transplanted in autoclaved pots containing 0.8% water–agar medium and kept in a growth chamber (day/night cycle: 14 h; 28 °C/10 h; 18 °C; relative humidity 60–70%; light intensity 1000 μmm^{-2} s^{-1} using natrium lamps). After attaining the two leaves stage, 10-μl from diluted supernatants of respective bacterial isolates was applied at the seedling apex. After a week, the shoot length, chlorophyll content, shoots' fresh and dry weights were recorded and compared with −ive and +ive controls. The *Burkholderia* sp. KCTC 11096BP strain [42] was used as the positive control while autoclaved DDW was used as a negative control. A novel bacterial isolate PEJ1011, which induced maximum plant growth in rice (data not shown), was selected for further study and re-streaked on fresh TSA medium. For long-term preservation, PEJ1011 was stored in 50% glycerol at −80 °C. PEJ1011 was checked for GAs production following standard procedure given in the section below. For GAs production, the PEJ1011 was grown in nutrient broth (NB) media for 3 days at 30 °C and 200 rpm.

2.2. Identification of GAs-producing PEJ1011

The isolate PEJ1011 was identified on the basis of partial 16S ribosomal RNA (rRNA) gene sequence. The total DNA was isolated following standard procedures [70]. The 16S rRNA gene was PCR amplified using the 27F primer (5′-AGAGTTTGATC(AC)TGGCTCAG-3′) and 1492R primer (5′-CGG (CT) TACCTTGTTACGACTT-3′), which were complementary to the 5′ end and 3′ end of the prokaryotic 16S rRNA, respectively [49]. The BLAST search program was used for nucleotide sequence homology of this bacterial isolate. The closely related sequences with the highest homology, query coverage and the lowest E values were selected and aligned by ClustalW using MEGA version 6.0software. *Bacillus thioparans* was used as outgroup during neighbour-joining tree generation using the same software. PEJ1011 showed maximum similarity for the subclade of *S. nematodiphila* (KC122708) and gave 72% bootstrap support when bootstrap 1K replications was used for the statistical support of the nodes in the phylogenetic tree. The 16S rRNA gene sequence of isolate PEJ1011 was submitted to NCBI GenBank under accession number KC819803.

2.3. PEJ1011 mediated plant growth promotion and cold stress resistance

We analysed *S. nematodiphila* PEJ1011 for its plant growth-