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Amelioration of non-irrigated stress and improvement of sweet pumpkin fruit quality by *Kushneria konosiri* endophytic bacteria

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Contribution to Environmental Biology

- We evaluated the alleviation of drought stress by bacteria in sweet pumpkin plants.
- *Kushenria konosiri* could be used as a biostimulant for the mitigation of drought stress and improvement of fruit quality.

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Abstract: This study examined the impact of two bacterial strains, H05E-12 and H05R-04, on alleviating non-irrigation-induced stress and its subsequent effects on the fruit productivity of sweet pumpkin plants. When subjected to non-irrigation-induced stress, the lipid peroxidation, proline, total phenol, and total soluble sugar content significantly decreased in plants treated with either H05E-12 or H05R-04 compared to the control. In a greenhouse experiment under non-irrigated conditions, H05E-12-treated plants exhibited higher stomatal conductance than the control, although there was no significant change in the soil plant analysis development (SPAD) value due to treatment. Upon re-watering, an increase in fruit diameter was observed in H05E-12-treated plants, and the L-ascorbic acid content in the fruit also showed a significant increase compared to the control. The H05E-12 strain was identified as *Kushneria konosiri*. To the best of our knowledge, this is the first report detailing the beneficial effects of *K. konosiri* on the alleviation of non-irrigation-induced stress and the promotion of plant growth in sweet pumpkin plants.

Keywords: Ascorbic acid, Biostimulant, *Kushneria konosiri*, Stress, Sweet pumpkin

1. INTRODUCTION

Recently, an increased interest towards food safety and sustainable plant management has encouraged several researchers to focus on the use of plant-associated microorganisms. The last few decades have witnessed the development of biological control approaches for disease suppression and the use of plant growth-promoting agents for productivity increase. With the move towards implementing more sustainable and environmentally friendly strategies, the concept of biostimulants has occurred in agriculture. Biostimulants, which include microbial and non-microbial substances, can ameliorate biotic and abiotic stresses by inducing tolerance, improving the nutrient-use efficiency in plants, and enhancing product quality (Rouphael and Colla 2020). Du Jardin (2015), and Colla and Rouphael (2015) define that plant biostimulants include any substance (chitosan, humic and fulvic acids, protein hydrolysate, phosphites, seaweed extracts, and silicon), microorganisms (arbuscular mycorrhizal fungi, plant growth-promoting rhizobacteria,

Trichoderma), and commercial products containing a mixture of substances and microorganisms that increases nutrient efficiency and abiotic stress tolerance, and enhances product quality traits.

Plant-associated microorganisms, belonging to the genera *Pseudomonas*, *Bacillus*, *Paenibacillus*, and *Streptomyces*, commonly generate plant-like hormones (auxin and cytokinin), antibiotic lipopeptides (iturin, surfactin, fengycin, nunamycin, nunapeptin, brasmycin, and braspeptin), polyketides (2,3-diacetylphloroglucinol, phenazine-1-carboxylic acid, phenazine-1-carboxamide, pyoluteorin, and pyrrolnitrin), volatiles, and various extracellular enzymes (glucanase, chitinase, and protease); prominently colonize and induce resistance in niche-grown host plants (Dimkić *et al.* 2022). Moreover, phosphatase solubilization, and indole-3-acetic acid and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production are important microbial traits associated with plant growth promotion (Toscano-Verduzco *et al.* 2020; Yuan *et al.* 2022). Plants cannot directly use insoluble soil phosphate; soil microbes help solubilize the insoluble phosphate by producing phosphatase and organic acids, thereby enhancing plant growth (Sang *et al.* 2011; Yoo *et al.* 2018). Kumar and Dubey (2022) reported that high concentrations of indole-3-acetic acid (IAA) and phosphate-solubilizing bacteria enhanced the vegetative growth of *Barleria lupulina* in a greenhouse test. Plant-associated bacteria also possess enzymes that hydrolyze ACC, which is a precursor for ethylene synthesis, and the ACC deaminase-producing bacteria exhibit plant growth-promoting activity under various abiotic stress conditions (Chandwani and Amaresan 2022). Therefore, these plant-associated bacteria, which are prominent as plant growth-promoting rhizobacteria (PGPR), are used as biofertilizers and biostimulants.

Pumpkin fruits provide a source of functional components such as free amino acids cysteine, arginine, and tyrosine, non-volatile organic acids, fatty acids such as oleic acid, linoleic acid, and palmitic acid; ascorbic acid (vitamin C) and carotenoid components. Among them, ascorbic acid in plants is involved in photosynthesis and cell growth control (Smirnoff and Wheeler 2000), and forms the first line of defense against damages induced by reactive oxygen species, and protects plant cells from various environmental and biological factors, such as oxidative stress, wounding, ozone, high salinity, and pathogen attacks (Boubakri 2017).

Sweet pumpkin in South Korea is grown in open fields from spring to summer and it can transiently suffer from a water-limited environment after transplanting during early spring. The spatial and temporal trend analysis of drought in South Korea (Azam *et al.* 2018) reported that drought occurred more frequently during late winter, early spring, and early autumn. Therefore, the sweet pumpkin plants could be transiently exposed to drought stress conditions in South Korea.

In this study, we used two bacterial strains, H05E-12, and H05R-04; investigated the effect on amelioration of non-irrigated stress and plant growth promotion, including productivity and ascorbic acid content as a functional component in sweet pumpkin.

2. MATERIALS AND METHODS

Two bacterial strains, H05E-12 and H05R-04, which were previously isolated and identified in our study (Kim *et al.* 2019), were utilized. The bacterial strains were cultured on tryptic soy agar (TSA, Difco, USA) at 28°C for 48 hours. Subsequently, a single colony from each strain was transferred to 200 mL of tryptic soy broth (TSB, Difco, USA) and incubated at 28°C with continuous shaking at 160 rpm for 48 hours. The resulting bacterial cultures were then centrifuged at 5,000 rpm for 15 minutes, and the harvested cell pellets were suspended in a 10 mM $MgSO_4$ solution. The suspensions were adjusted to an optical density (OD) of 0.2 at 600 nm, corresponding to a cell concentration of 1×10^8 cells mL^{-1}, and these suspensions were used for the subsequent plant assays.

For the plant assay, 'Minimom' sweet pumpkin seedlings (NongHyup Seed Corp., Korea) were initially grown in a 72-hole tray filled with potting mixture from Bunong, South Korea. Seedlings at the first-leaf stage were transplanted into plastic pots with a 12 cm diameter, which contained pasteurized field soil. These plants were grown until they reached the fourth-leaf stage. One day prior to inducing non-irrigated stress, the plants were thoroughly saturated with tap water. After this, water was withheld from the plants for a duration of five days, during which the mean temperature was maintained at 28 ± 2°C, with a relative humidity range of 50−60%. Following the non-irrigation period, the leaves of the sweet pumpkin plants were flash-frozen using liquid nitrogen and stored in a deep

freezer at −70°C. Subsequently, the plant tissue samples were homogenized using a homogenizer (Retsch MM200, Germany).

The assessment of various biochemical parameters in the leaves included the measurement of malondialdehyde (MDA) content, proline content, total phenolic content (TPC), and total soluble sugar (TSS) content. MDA content was determined according to the method outlined by Dhindsa *et al.* (1981). Crushed samples (0.1 g) were homogenized in 0.5 mL of 0.1% trichloroacetic acid (TCA) and then centrifuged at 13,000 rpm for 10 minutes. After mixing the separated supernatant (0.2 mL) with 0.6 mL of 20% TCA containing 0.5% 2-thiobarbituric acid (TBA), the reaction mixture was allowed to incubate at 90°C for 30 minutes in a water bath and then cooled on ice for 5 minutes. The absorbance was measured at 450, 532, and 600 nm using a spectrophotometer (Infinite M200 PRO; Tecan, Switzerland). The MDA content was calculated following the method described by Bao *et al.* (2009). Proline content was measured based on the procedure described by Bates *et al.* (1973). Grinded samples (0.1 g) were added to 1.2 mL of 3% aqueous sulfosalicylic acid and centrifuged at 13,000 rpm for 10 minutes. The transferred supernatant was then mixed with 0.5 mL of glacial acetic acid, followed by the addition of 1 mL of acid ninhydrin reagent. After thorough mixing, the mixture was incubated at 90°C for 1 hour and subsequently cooled on ice. The reaction mixture was then combined with an equal amount of toluene, vortexed for 30 seconds, and the absorbance was measured at 520 nm in the upper layer using a spectrophotometer. Proline content was calculated using a standard curve. TPC in sweet pumpkin leaves was assessed using Folin-Ciocalteu reagent and calculated using a standard curve prepared with gallic acid (Tinyane *et al.* 2013). The extraction buffer (acetone:water, 1 : 1, v/v) was prepared at a ratio of 1 mL per 0.1 g of the sample and vortexed for 1 hour. 9 μL of supernatant was added to 109 μL of Folin-Ciocalteu reagent and allowed to react for 3 minutes at room temperature. The resulting mixture was combined with 180 μL of Na_2CO_3 solution and incubated at 50°C for 5 minutes in a water bath, followed by cooling to room temperature. The reaction was then measured at 760 nm using a spectrophotometer. TSS was determined using the anthrone-sulfuric reagent method (Yemm and Willis 1954). 0.1 g of leaf sample was mixed with extraction buffer (methanol:chloroform:water, 60 : 25 : 15, v/v) and incubated at 60°C for 2 hours in a water bath. Subsequently, the mixture was centrifuged at 10,000 rpm for 20 minutes. The supernatant was then combined with 1 mL of anthrone-sulfuric reagent and incubated at 95°C for 15 minutes. After cooling to room temperature, the absorbance was measured at 625 nm using a spectrophotometer. TSS was calculated based on a standard curve for glucose. Each of these parameters, including MDA, proline, TPC, and TSS, were evaluated twice with five replicates for each analysis.

In the plastic house assay, sweet pumpkin plants at the third-leaf stage were transplanted into Wagner pots with a diameter of 25 cm and a depth of 30 cm, which contained 2 kg of field soil. The pots were subjected to irrigation with tap water five times a day, each for a duration of 7 minutes, at a pressure of 2.5 bars (0.25 MPa) using one line per pot. The pots were arranged in a completely randomized block design with three replicates. The decision regarding the duration of non-irrigation, which was set at two days with a mean temperature of 24.7°C and a relative humidity of 62%, was based on the stomatal conductance value (the control value was less than 60). Stomatal conductance and soil plant analysis development (SPAD) values of the sweet pumpkin leaves were determined using a SC-1 leaf porometer (Decagon Devices, Pullman, WA, USA) and a SPAD value meter (Minolta Co. Ltd., Japan), respectively. After the non-irrigation period, the pots were subsequently irrigated with water five times a day for 7 minutes, with one line per pot, at a pressure of 0.25 MPa. Thirty days after re-watering, a portion of the sweet pumpkin was harvested and freeze-dried at −40°C and 0.100 mbar for a duration of 7 days, while another 5 g portion of fresh sweet pumpkin was juiced and the Brix value was measured using a refractometer (PAL-3; Atago Co., Ltd., Japan).

The ascorbic acid content in sweet pumpkins was analyzed using high-performance liquid chromatography (HPLC) following the method by Zhang and Hamauzu (2003). Freeze-dried sample (0.1 g) was homogenized with 1.5 mL of 5% metaphosphoric acid and centrifuged at 4,000 rpm for 10 minutes. The resulting supernatant was filtrated through a PVDF syringe filter (0.45 μm, PALL Life Sciences, USA). The chromatographic column was a YMC-Pack Polyamine II (250 × 4.6 mm, 5 μm) maintained at 25°C, with the mobile phase consisting of (A) 0.1% phosphoric acid

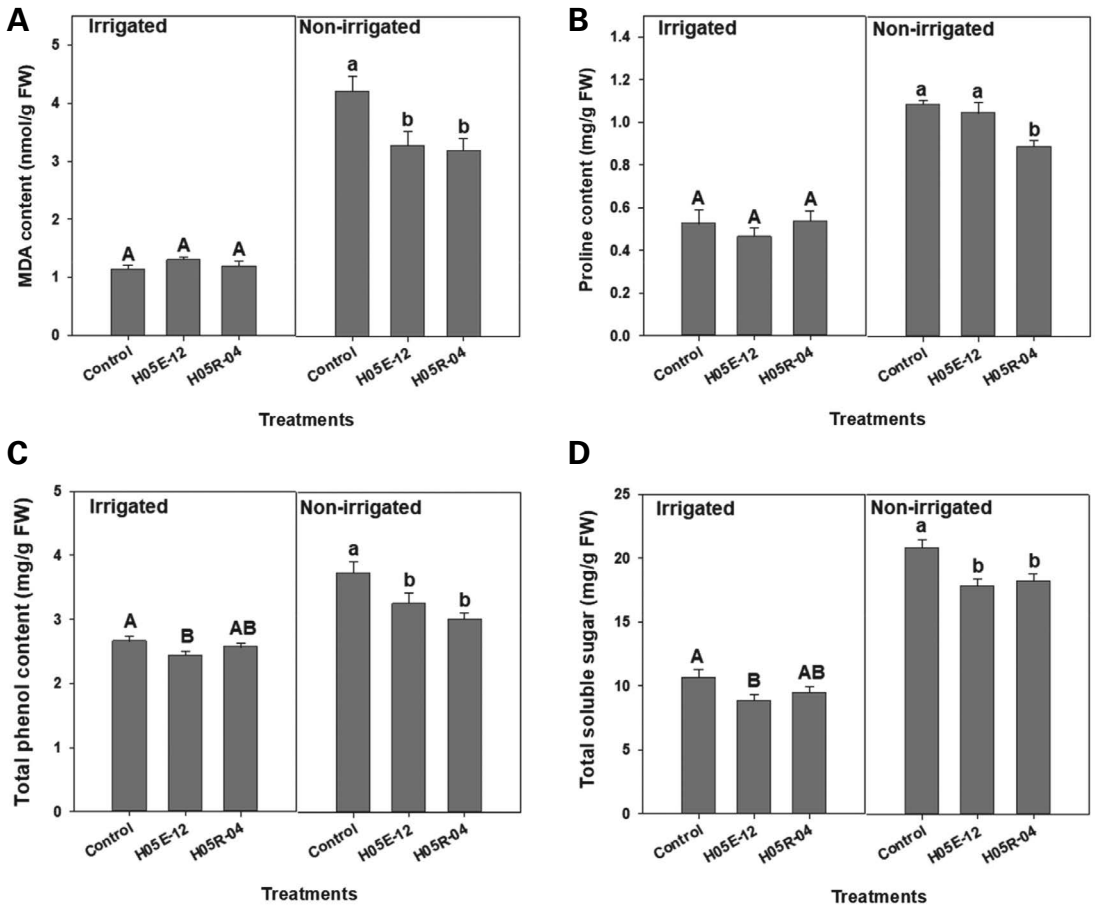

Fig. 1. Malondialdehyde (A), proline (B), total phenol (C), and total soluble sugar content (D) in the leaves of sweet pumpkin plants treated with strains H05E-12 and H05R-04. Non-irrigation was conducted for five days in the pot assay. Letters on the bars indicate statistical differences by LSD ($p<0.05$), and error bars indicate standard errors.

(in water) and (B) acetonitrile. The elution condition involved isocratic elution for 0–10 minutes with 75% B. The flow rate was 1 mL min^{-1}, and the injection volume was 10 µL. The parameters of photodiode array (PDA) detector (2998; Waters, USA) were set to detected at 254 nm. L-ascorbic acid standards were used to create standard curves at concentrations of 1, 10, 50, 100, 200, and 500 µg mL^{-1}. The L-ascorbic acid content and Brix were determined with three replicates, each comprising three plants.

To construct a phylogenetic tree based on 16S rRNA sequences, genomic DNA was extracted from the bacteria using the G-spinTM Genomic DNA extraction kit (iNtRON, Korea). Amplification of 16S rRNA was carried out with a universal primer set, 785F (5-GGA TTA GAT ACC CTG GTA-3) and 907R (5-CCG TCA ATT CMT TTR AGT TT-3). The resulting amplicons were sequenced by Macrogen Co. (Seoul, Korea), and the obtained sequences were compared to those of type strains using the EzBioCloud database. Phylogenetic analysis was performed using the Molecular Evolutionary Genetics Analysis (MEGA 7.0) with the neighbor-joining statistical method, with 1,000 bootstrap values.

Statistical analysis of the data was conducted using the Statistical Analysis System (SAS) software (version 9.4, SAS Institute Inc., Cary, NC, USA). Data from repeated experiments were combined after confirming the homogeneity of variances using Levene's test, and subsequent statistical analysis was performed. Analysis of variance was carried out, and the means were statistically differentiated using the least significant difference (LSD) test with a significance level set at $p<0.05$.

3. RESULTS

3.1. Alleviative activity of the bacterial strains against non-irrigated stress in sweet pumpkin plants

In our non-irrigation system, the stressed plants exhibited significant increases in MDA content (F= 200.33, $p<0.0001$), as well as elevated levels of proline (F=164.84, $p<0.0001$), TPC (F=53.07, $p<0.0001$), and TSS (F=300.66, $p<0.0001$) in their leaves when compared to irrigated plants. Towards the end of the non-irrigation period, the MDA content, associated with lipid peroxidation, as well as TPC and TSS, showed significant reductions in the bacterial-treated plants compared to the control. Proline content also significantly decreased in H05R-04-treated plants (Fig. 1). Under irrigated conditions, lipid peroxidation and proline content in the plants were not affected by bacterial treatment. However, TPC and TSS levels decreased in H05E-12-treated plants compared to those in the control (Fig. 1).

In our plastic house assay, treatment with H05E-12 alleviated the decline in stomatal conductance caused by non-irrigated stress when compared to the control (F=6.64, p=0.0494). However, SPAD values, which are related to chlorophyll content and overall greenness, remained unaffected by bacterial treatment (F=1.42, p=0.3143) (Fig. 2).

3.2. Effect of bacterial treatments on productivity of sweet pumpkin

The sweet pumpkin fruits were harvested 30 days after the non-irrigation period, followed by re-watering. In H05RE-12-treated plants, the fruit diameter per plant showed a significant increase compared to the control plants (Table 1). However, under the irrigation system, bacterial treatments did not significantly impact productivity (Table 1). The sugar levels in fruits harvested from H05E-12-treated plants, as measured using a Brix refractometer, were similar to those in the control, regardless of the presence of stress (Table 1).

L-ascorbic acid content tended to accumulate in the fruits when subjected to non-irrigated stress (F=27.04, p=0.01). However, L-ascorbic acid was found to accumulate in the fruits of bacteria-treated plants regardless of the presence of stress, with both H05R-04 (F=0.05, p=0.8269) and H05E-12 (F=0.33, p=0.6063) (Table 1). Notably, the treatment with strain H05E-12 resulted in a significant increase in L-ascorbic acid in the stressed plants (Table 1).

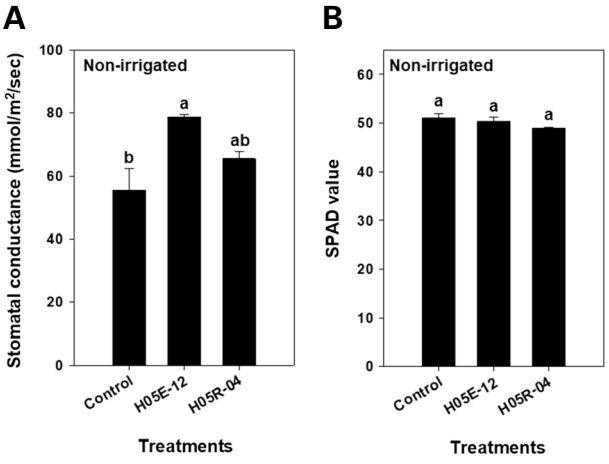

Fig. 2. Stomatal conductance (A) and SPAD value (B) of the leaves of sweet pumpkin plants in a plastic house. One week after bacterial treatment, non-irrigation was conducted for two days. Letters on the bars indicate statistical differences by LSD ($p<0.05$), and error bars indicate standard errors.

Table 1. Productivity, brix, and L-ascorbic acid content of sweet pumpkin treated with bacterial strains.

Condition	Treatments	Fruit weight/plants (g)	Fruit diameter/plants (mm)	Brix (%)	L-ascorbic acid (μg g^{-1})
Irrigated	Control	291.19±5.25 a*	95.95±1.34 a	9.51±0.76 a	285.65±25.71 b
	H05E-12	327.24±20.07 a	100.03±2.09 a	11.32±0.38 a	533.16±31.67 a
	H05R-04	301.92±23.09 a	95.83±1.45 a	9.82±0.53 a	505.18±9.22 a
Non-irrigated and re-watered	Control	324.92±12.92 a	98.03±0.78 b	9.63±0.20 ab	458.55±2.65 b
	H05E-12	415.17±48.83 a	107.78±4.46 a	10.93±0.90 a	557.16±11.88 a
	H05R-04	338.41±8.69 a	99.49±1.18 a	8.97±0.23 b	498.04±29.18 ab

*Small letters indicate significant differences between treatments in each condition by LSD ($p<0.05$).

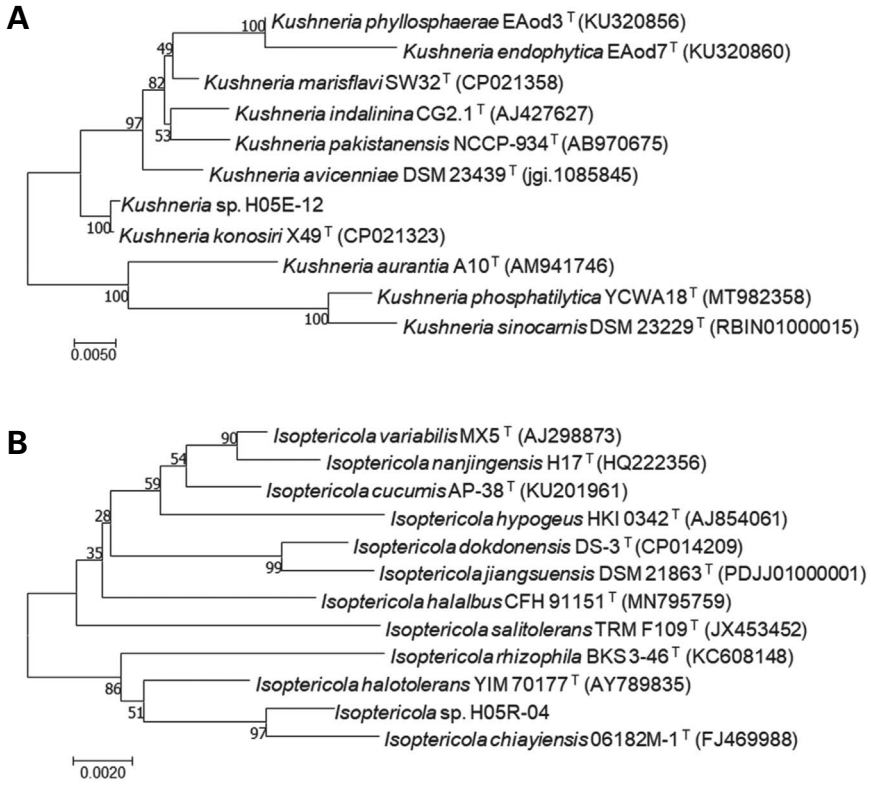

Fig. 3. Phylogenetic trees of (A) H05E-12 and (B) H05R-04 were constructed by neighbor-joining analysis based on comparisons of 16S rRNA sequences. Bootstrap values = 1,000 replicates.

3.3. Identification of bacterial strains that alleviated non-irrigated stress in sweet pumpkin

The two strains, H05R-04, and H05E-12, were identified as follows: H05R-04 showed a similarity of 99.3% with a coverage of 100% and was related to *Isoptericola chiayiensis* 06182M-1. H05E-12 exhibited an even higher similarity of 99.9% with a coverage of 100% and was related to *Kushneria konosiri* X49 (Fig. 3).

4. DISCUSSION

In this study, we have successfully demonstrated that H05E-12, identified as *Kushneria konosiri*, possesses the potential to alleviate non-irrigated stress in sweet pumpkin plants, enhance fruit productivity, and increase the content of L-ascorbic acid compared to the control. Managing plants in conditions of water stress has gained greater significance due to the effects of worldwide climate change. According to the IPCC (2014), climate change was caused by the increasing concentration of CO_2. The elevated CO_2 concentration was affected by global warming in the range of 1.0–3.7°C (IPCC 2014). Additionally, climate change was anticipated to have disrupted rainfall patterns, increasing the frequency of droughts (Ben Mariem *et al.* 2021; Chaudhry and Sidhu 2022). To mitigate this issue, research has focused on plant breeding and genetic modification aimed at reducing water requirements and enhancing water usage efficiency (Acreche 2017; Farooq *et al.* 2019; Mega *et al.* 2019). An alternative approach involves leveraging plant-associated bacteria to alleviate various abiotic stresses, such as drought, temperature variations, and salinity (Kim *et al.* 2019; Shin *et al.* 2019; Yoo *et al.* 2019).

The tolerance of stress through bacteria-based methods typically involves the accumulation of osmotolerant compounds like proline, trehalose, and betaine, the regulation of hormonal processes related to water transpiration and root structure, and the adjustment

of plant elements such as sodium, potassium, and calcium. Marulanda *et al.* (2009) demonstrated that *Pseudomonas putida* and *Bacillus megaterium* displayed osmotic tolerance, accumulating proline during NaCl-induced osmotic stress, increasing indole-3-acetic acid (IAA) production under PEG-induced drought stress, and promoting plant growth. Similarly, Kasim *et al.* (2013) proposed that plants primed with bacteria exhibited improved homeostatic mechanisms, including the attenuation of stress-related gene expression and reduced damage from drought stress in wheat. On the other hand, the alleviation of drought stress exhibited a different response compared to drought tolerance. Gontia-Mishra *et al.* (2016) reported that PGPR strains, including *Klebsiella* sp. IG 3, *Enterobacter Iudwigii* IG 10, and *Flavobacterium* sp. IG 15, were found to mitigate drought stress. These strains maintained relative water contents (RWC) and reduced proline, TSS, H_2O_2, electrolyte leakage, and MDA. Additionally, they down-regulated stress-related genes such as *CAT1* and *DREB2A*. Moreover, Kasim *et al.* (2021) indicated protection against damage under drought stress by *Azospirillum brasilense* NO40 and *Stenotrophomonas maltophilia* B11. These strains increased RWC, while decreasing electrolyte leakage, MDA, H_2O_2, proline, and antioxidant enzyme activities. Additionally, Curá *et al.* (2017) demonstrated an induced increase in RWC, and reduction in proline, MDA, ethylene, and abscisic acid (ABA) by treated with *Azospirillum brasilense* SP-7 and *Herbaspirillum seropedicae* Z-152. In our study, we observed an increase in stomatal conductance in H05E-12 treatment, suggesting a potential decrease in ABA content. ABA was regulated stomatal closure (Li *et al.* 2020). Stomatal conductance indirectly indicated plant water status and stress and correlating transpiration (Martínez-Vilalta and Garcia-Forner 2017; Querejeta *et al.* 2022). Under drought conditions, both stomatal conductance and transpiration rate were reduced, limiting ambient CO_2 diffusion and inhibiting photosynthetic rate (Ahmad *et al.* 2019; Wang *et al.* 2019; Tiwari *et al.* 2020).

In a prior investigation, *B. siamensis* H30-3 produced extracellular polysaccharides and preserved soil moisture content when cabbage was grown under drought and high-temperature stress conditions (Shin *et al.* 2019). *B. mesonae* H20-5 enhanced plant salinity tolerance by modifying plant physiological responses, including proline accumulation, increased calcium ion levels, and up-regulated expression of hormone-related genes (Yoo *et al.* 2019). Additionally, *B. butanolivorance* KJ40 induced drought tolerance in pepper plants (Kim *et al.* 2019). In the current study, *K. konosiri* H05E-12 reduced lipid peroxidation in sweet pumpkin plants under non-irrigated stress, suggesting that this strain might have mitigated the initial stress and moderated plant responses associated with drought, including the accumulation of proline, soluble sugars, and phenolic compounds.

In the current study, sweet pumpkin plants treated with H05E-12 and subjected to non-irrigated stress exhibited a notable increase in ascorbic acid content. Ascorbic acid contains a diverse array of phenolic compounds and contributes to the provision of antioxidant properties. In a study by Rahman *et al.* (2018), it was observed that *B. amyloliquefaciens* BChi1 and *Paraburkholderia fungorum* BRRh-4 enhanced various antioxidant components such as total antioxidants, carotenoids, flavonoids, phenolics, and total anthocyanins in strawberries. Morais *et al.* (2019) reported that *Pedobacter* sp. CC1 expedited strawberry maturation and boosted the accumulation of total phenolics and flavonoids.

However, under certain environmental constraints, such as limited nitrogen availability, the inoculation of *Azospirillum brasilense* M3 and *Pantoea dispersa* C3 led to increased concentrations of citric acid, ascorbic acid, and succinic acid in green sweet peppers (del Amor *et al.* 2008). In a previous study, the salinity-tolerant bacterium *B. mesonae* H20-5 was found to enhance polyphenol, lycopene, and total soluble solids in tomato plants when compared to untreated plants under salinity stress conditions (Yoo *et al.* 2019). These findings suggest that plant-associated bacteria can induce alterations in plant physiology and the composition of plant metabolites related to fruit quality and plant growth. These changes may be linked to the acceleration of plant ripening by the treated strains.

Generally, the total soluble solids and sugar content in fruits tend to increase with the progression of plant growth. Jakopic *et al.* (2007) reported a significant increase in citric acid concentration during fruit ripening. Del Amor *et al.* (2008) noted that the organic acid concentration in sweet pepper varied with the fruit's developmental stage. For example, in ripened yellow sweet peppers, citric acid and ascorbic acid levels increased, while succinic acid decreased during

ripening. Gnayfeed *et al.* (2001) observed that ascorbic acid reached its peak at the color break stage, which occurs during ripening. Sharma and Rao (2013) revealed that pumpkin fruits accumulated ascorbic acid, carotenoids, and carbohydrates during ripening, and starch degradation occurred alongside an increase in reducing sugars and total sugars. Moreover, Morais *et al.* (2019) demonstrated that *Pedobacter* spp. CC1 significantly accelerated strawberry maturation, resulting in strawberries being harvested two weeks earlier in the inoculated plants compared to the non-inoculated ones. Therefore, the results in our study may also be attributed to the stimulation of plant development in sweet pumpkin plants treated with H05E-12.

Strain H05E-12 was identified as *Kushneria konosiri*, originally isolated from Korean traditional salt-fermented seafood. This strain is characterized as halophilic, strictly aerobic, and gram-negative, as reported by Yun *et al.* (2017). The Kushneria genus is known for its halophilic and phosphorus-solubilizing microorganisms (Zhu *et al.* 2011), with *K. marisflavi* CSE9, a halotolerant endophyte, having the ability to mitigate salt stress and promote plant growth in saline environments, as demonstrated by Szymańska *et al.* (2020). Additionally, other species within the *Kushneria* genera, such as *K. phyllosphaerae* and *K. endophytica*, have been identified as plant growth-promoting endophytes (Navarro-Torre *et al.* 2018), and *K. phosphatilytica* has been recognized as a phosphate-solubilizing bacterium (Du *et al.* 2021). Recently, Yang *et al.* (2023) reported that *Acivennia marina*, such as salt-tolerance plants, isolated *K. konosiri* 1-1 and *B. marisflavi* 23-1 in phyllosphere and rhizosphere, respectively. The *K. konosiri* 1-1 and *B. marisflaci* 23-1 strains promoted growth under salt conditions.

In the context of this study, we have observed that *K. konosiri* H05E-12 plays a role in alleviating water stress and increasing the L-ascorbic acid content in sweet pumpkin plants. In light of these findings, we have chosen *K. konosiri* H05E-12 as a potential biostimulant agent for mitigating non-irrigated stress and enhancing plant productivity. Furthermore, a significant increase in ascorbic acid content was observed in the fruits of sweet pumpkin plants. To the best of our knowledge, this study represents the first report of the biostimulant potential of *K. konosiri*, particularly in its capacity to alleviate non-irrigated stress and promote plant growth in sweet pumpkin plants.

CRediT authorship contribution statement

ST Kim: Methodology, Investigation, Visualization, Data Curation, Writing - Original draft. **MK Sang:** Validation, Conceptualization, Writing - Review & editing, Supervision.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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REFERENCES

Acreche MM. 2017. Nitrogen-, water-and radiation-use efficiencies affected by sugarcane breeding in Argentina. Plant Breed. 136:174–181. https://doi.org/10.1111/pbr.12440

Ahamd S, M Kamran, R Ding, X Meng, H Wang, I Ahmad, S Fahad and Q Han. 2019. Exogenous melatonin confers drought stress by promoting plant growth, photosynthetic capacity and antioxidant defense system of maize seedlings. PeerJ 7:e7793. https://doi.org/10.7717/peerj.7793

Azam M, SJ Maeng, HS Kim, SW Lee and JE Lee. 2018. Spatial and temporal trend analysis of precipitation and drought in South Korea. Water 10:765. https://doi.org/10.3390/w10060765

Bao AK, SM Wang, GQ Wu, JJ Xi, JL Zhang and CM Wang. 2009. Overexpression of the arabidopsis H^+ -PPase enhanced resistance to salt and drought stress in transgenic alfalfa (*Medicago sativa* L.). Plant Sci. 176:232–240. https://doi.org/10.1016/j.plantsci.2008.10.009

Bates LS, RP Waldren and ID Teare. 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207. https://doi.org/10.1007/BF00018060

Ben Mariem S, D Soba, B Zhou, I Loladze, F Morales and I Aranjuelo. 2021. Climate change, crop yield, and grain quality of C_3 cereals: A meta-analysis of [CO_2], temperature, and drought effects. Plants 10:1052. https://doi.org/10.3390/plants10061052

Boubakri H. 2017. The role of ascorbic acid in plant-patho-

gen interactions. pp. 255–271. In: Ascorbic Acid in Plant Growth, Development and Stress Tolerance (Hossain M, S Munné-Bosch, D Burritt, P Diaz-Vivancos, M Fujita and A Lorence, eds.). Springer. Cham, Switzerland. https://doi.org/10.1007/978-3-319-74057-7_10

Chandwani S and N Amaresan. 2022. Role of ACC deaminase producing bacteria for abiotic stress management and sustainable agriculture production. Environ. Sci. Pollut. Res. Int. 29:22843–22859. https://doi.org/10.1007/s11356-022-18745-7

Chaudhry S and GPS Sidhu. 2022. Climate change regulated abiotic stress mechanisms in plants: A comprehensive review. Plant Cell Reports 41:1–31. https://doi.org/10.1007/s00299-021-02759-5

Colla G and Y Rouphael. 2015. Biostimulants in horticulture. Sci. Hortic. 196:1–2. https://doi.org/10.1016/j.scienta.2015.10.044

Curá JA, DR Franz, JE Filosofía, KB Balestrasse and LE Burgueño. 2017. Inoculation with *Azospirillum* sp. and *Herbaspirillum* sp. bacteria increases the tolerance of maize to drought stress. Microorganisms 5:41. https://doi.org/10.3390/microorganisms5030041

del Amor FM, A Serrano-Martinez, MI Fortea, P Legua and E Núñez-Delicado. 2008. The effect of plant-associative bacteria (*Azospirillum* and *Pantoea*) on the fruit quality of sweet pepper under limited nitrogen supply. Sci. Hortic. 117:191–196. https://doi.org/10.1016/j.scienta.2008.04.006

Dhindsa RS, P Plumb-Dhindsa and TA Thorpe. 1981. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot. 32:93–101. https://doi.org/10.1093/jxb/32.1.93

Dimkić I, T Janakiev, M Petrović, G Degrassi and D Fira. 2022. Plant-associated bacillus and pseudomonas antimicrobial activities in plant disease suppression via biological control mechanisms - A review. Physiol. Mol. Plant Pathol. 117:101754. https://doi.org/10.1016/j.pmpp.2021.101754

Du G, L Qu, X Hong, C Li, D Ding, P Gao and Q Xu. 2021. *Kushneria phosphatilytica* sp. nov., a phosphate-solubilizing bacterium isolated from a solar saltern. Int. J. Syst. Evol. Microbiol. 71:004619. https://doi.org/10.1099/ijsem.0.004619

Du Jardin P. 2015. Plant biostimulants: Definition, concept, main categories and regulation. Sci. Hortic. 196:3–14. https://doi.org/10.1016/j.scienta.2015.09.021

Farooq M, M Hussain, S Ul-Allah and KH Siddique. 2019. Physiological and agronomic approaches for improving water-use efficiency in crop plants. Agric. Water Manage. 219:95–108. https://doi.org/10.1016/j.agwat.2019.04.010

Gnayfeed MH, HG Daood, PA Biacs and CF Alcaraz. 2001. Content of bioactive compounds in pungent spice red pepper (paprika) as affected by ripening and genotype. J. Sci. Food Agric. 81:1580–1585. https://doi.org/10.1002/jsfa.982

Gontia-Mishra I, S Sapre, A Sharma and S Tiwari. 2016. Amelioration of drought tolerance in wheat by the interaction of plant growth-promoting rhizobacteria. Plant Biol. 18:992–1000. https://doi.org/10.1111/plb.12505

IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II, and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (The Core Writing Team, RK Pachauri and L Meyer eds.). The Intergovernmental Panel on Climate Change. Geneva, Switzerland.

Jakopic J, R Veberic and F Stampar. 2007. The effect of reflective foil and hail nets on the lighting, color and anthocyanins of 'Fuji' apple. Sci. Hortic. 115:40–46. https://doi.org/10.1016/j.scienta.2007.07.014

Kasim WA, ME Osman, MN Omar, IA Abd El-Daim, S Bejai and J Meijer. 2013. Control of drought stress in wheat using plant-growth-promoting bacteria. J. Plant Growth Regul. 32:122–130. https://doi.org/10.1007/s00344-012-9283-7

Kasim WA, MEH Osman, MN Omar and S Salama. 2021. Enhancement of drought tolerance in *Triticum aestivum* L. seedling using *Azospirillum brasilense* NO40 and *Stenotrophomonas maltophilia* B11. Bull. Natl. Res. Cent. 45:95. https://doi.org/10.1186/s42269-021-00546-6

Kim ST, S Yoo, J Song, H Weon and MK Sang. 2019. Screening of bacterial strains for alleviating drought stress in chili pepper plants. Res. Plant Dis. 25:136–142. https://doi.org/10.5423/RPD.2019.25.3.136

Kumar N and RC Dubey. 2022. Plant growth-promoting attributes of an endophyte *Enterobacter roggenkampii* BLS02 isolated from *Barleria lupulina* Lindl. Org. Agric. 12:137–145. https://doi.org/10.1007/s13165-021-00375-x

Li S, X Li, Z Wei and F Liu. 2020. ABA-mediated modulation of elevated CO_2 on stomatal response to drought. Curr. Opin. Plant Biol. 56:174–180. https://doi.org/10.1016/j.pbi.2019.12.002

Martínez-Vilalta J and N Garcia-Forner. 2017. Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydric concept. Plant Cell Environ. 40:962–976. https://doi.org/10.1111/pce.12846

Marulanda A, J Barea and R Azcón. 2009. Stimulation of plant growth and drought tolerance by native microorganisms (AM fungi and bacteria) from dry environments: Mechanisms related to bacterial effectiveness. J. Plant Growth Regul. 28:115–124. https://doi.org/10.1007/s00344-009-9079-6

Mega R, F Abe, JS Kim, Y Tsuboi, K Tanaka, H Kobayashi, Y Sakata, K Hanada, H Tsujimoto, J Kikuchi, SR Cutler and M Okamoto. 2019. Tuning water-use efficiency and drought

tolerance in wheat using abscisic acid receptors. Nat. Plants 5:153–159. https://doi.org/10.1038/s41477-019-0361-8

Morais MC, Â Mucha, H Ferreira, B Gonçalves, E Bacelar and G Marques. 2019. Comparative study of plant growth-promoting bacteria on the physiology, growth and fruit quality of strawberry. J. Sci. Food Agric. 99:5341–5349. https://doi.org/10.1002/jsfa.9773

Navarro-Torre S, L Carro, ID Rodríguez-Llorente, E Pajuelo, MÁ Caviedes, JM Igual, S Redondo-Gómez, M Camacho, HP Klenk and MDC Montero-Calasanz. 2018. *Kushneria phyllosphaerae* sp. nov. and *Kushneria endophytica* sp. nov., plant growth promoting endophytes isolated from the halophyte plant *Arthrocnemum macrostachyum*. Int. J. Syst. Evol. Microbiol. 68:2800–2806. https://doi.org/10.1099/ijsem.0.002897

Querejeta JI, I Prieto, C Armas, F Casanoves, JS Diémé, M Diouf, H Yossi, B Kaya, FI Pugnaire and GM Rusch. 2022. Higher leaf nitrogen content is linked to tighter stomatal regulation of transpiration and more efficient water use across dryland trees. New Phytol. 235:1351–1364. https://doi.org/10.1111/nph.18254

Rahman M, AA Sabir, JA Mukta, MMA Khan, M Mohi-Ud-Din, MG Miah, M Rahman and MT Islam. 2018. Plant probiotic bacteria *Bacillus* and *Paraburkholderia* improve growth, yield and content of antioxidants in strawberry fruit. Sci. Rep. 8:2504. https://doi.org/10.1038/s41598-018-20235-1

Rouphael Y and G Colla. 2020. Editorial: biostimulants in agriculture. Front. Plant Sci. 11:40. https://doi.org/10.3389/fpls.2020.00040

Sang MK, JD Kim, BS Kim and KD Kim. 2011. Root treatment with rhizobacteria antagonistic to Phytophthora blight affects anthracnose occurrence, ripening, and yield of pepper fruit in the plastic house and field. Phytopathology 101:666–678. https://doi.org/10.1094/PHYTO-08-10-0224

Sharma S and TV Ramana Rao. 2013. Nutritional quality characteristics of pumpkin fruit as revealed by its biochemical analysis. Int. Food Res. J. 20:2309–2316

Shin DJ, S Yoo, JK Hong, H Weon, J Song and MK Sang. 2019. Effect of *Bacillus aryabhattai* H26-2 and *B. siamensis* H30-3 on growth promotion and alleviation of heat and drought stresses in Chinese cabbage. Plant Pathol. J. 35:178–187. https://doi.org/10.5423/PPJ.NT.08.2018.0159

Smirnoff N and GL Wheeler. 2000. Ascorbic acid in plants: Biosynthesis and function. Crit. Rev. Plant Sci. 19:267–290. https://doi.org/10.1080/07352680091139231

Szymańska S, J Tyburski, A Piernik, M Sikora, J Mazur and H Katarzyna. 2020. Raising beet tolerance to salinity through bioaugmentation with halotolerant endophytes. Agronomy 10:1571. https://doi.org/10.3390/agronomy10101571

Tinyane PP, D Sivakumar and P Soundy. 2013. Influence of photo-selective netting on fruit quality parameters and bioactive compounds in selected tomato cultivars. Sci. Hortic. 161:340–349. https://doi.org/10.1016/j.scienta.2013.06.024

Tiwari RK, MK Lal, R Kumar, KN Chourasia, KC Naga, D Kumar, SK Das and G Zinta. 2020. Mechanistic insights on melatonin-mediated drought stress mitigation in plants. Physiol. Plant. 172:1212–1226. https://doi.org/10.1111/ppl.13307

Toscano-Verduzco FA, PA Cedeño-Valdivia, W Chan-Cupul, HA Hernández-Ortega, E Ruiz-Sánchez, E Galindo-Velasco and E Cruz-Crespo. 2020. Phosphates solubilization, indol-3-acetic acid and siderophores production by *Beauveria brongniartii* and its effect on growth and fruit quality of *Capsicum chinense*. J. Horticult. Sci. Biotechnol. 95:235–246. https://doi.org/10.1080/14620316.2019.1662737

Wang J, J Chen, A Sharma, S Tao, B Zheng, M Landi, H Yuan and D Yan. 2019. Melatonin stimulates activities and expression level of antioxidant enzymes and preserves functionality of photosynthetic apparatus in Hickory plants (*Carya cathayensis* Sarg.) under PEG-promoted drought. Agronomy 9:702. https://doi.org/10.3390/agronomy9110702

Yang X, Z Dai, R Yuan, Z Guo, H Xi, Z He and M Wei. 2023. Effects of salinity on assembly characteristics and function of microbial communities in the phyllosphere and rhizosphere salt-tolerant *Avicennia marina* mangrove species. Microbiol. Spectr. 11:e03000-22. https://doi.org/10.1128/spectrum.03000-22

Yemm EW and A Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57:508–514. https://doi.org/10.1042/bj0570508

Yoo SJ, DJ Shin, HY Won, J Song and MK Sang. 2018. *Aspergillus terreus* JF27 promotes the growth of tomato plants and induces resistance against *Pseudomonas syringae* pv. *tomato*. Mycobiology 46:147–153. https://doi.org/10.1080/12298093.2018.1475370

Yoo SJ, HY Weon, J Song and MK Sang. 2019. Induced tolerance to salinity stress by halotolerant bacteria *Bacillus aryabhattai* H19-1 and *B. mesonae* H20-5 in tomato plants. J. Microbiol. Biotechnol. 29:1124–1136. https://doi.org/10.4014/jmb.1904.04026

Yuan Y, M Zu, L Sun, J Zuo and J Tao. 2022. Isolation and screening of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase producing PGPR from *Paeonia lactiflora* rhizosphere and enhancement of plant growth. Sci. Hortic. 297:110956. https://doi.org/10.1016/j.scienta.2022.110956

Yun JH, SK Park, JY Lee, MJ Jung and JW Bae. 2017. *Kushneria konosiri* sp. nov., isolated from the Korean salt-fermented seafood Daemi-jeot. Int. J. Syst. Evol. Microbiol. 67:3576–3582. https://doi.org/10.1099/ijsem.0.002170

Zhang D and Y Hamauzu. 2003. Phenolic compounds, ascorbic acid, carotenoids and antioxidant properties of green, red and yellow bell peppers. J. Food Agric. Environ. 1:22–27.

Zhu F, L Qu, X Hong and X Sun. 2011. Isolation and characterization of a phosphate-solubilizing halophilic bacterium *Kushneria* sp. YCWA18 from Daqiao Saltern on the coast of yellow sea of China. Evid.-based Complement Altern. Med. 2011:615032. https://doi.org/10.1155/2011/615032