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Research Article

PGPR alleviated the negative effects of low temperature and low light stress on the growth and physiology of violet plants

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Abstract

Plants in greenhouses are often exposed to low temperature and low light conditions in the winter, and this has a major effect on flower cultivation. Inoculation of plant growth-promoting rhizobacteria (PGPR) can improve the growth of plants under stress. Here, we examined the effects of *Pseudomonas fluorescens* on the growth, photosynthesis, chlorophyll fluorescence parameters, and antioxidant enzyme systems of violet plants (*Matthiola incana*) exposed to normal temperature and normal light intensity (18 °C/11 °C, 500 μmol·m^{-2}·s^{-1}, NN), low temperature and normal light intensity (4 °C/1 °C, 500 μmol·m^{-2}·s^{-1}, LN), normal temperature and low light intensity (18 °C/11°C, 100 μmol·m^{-2}·s^{-1}, NL), and low temperature and low light intensity (4 °C/1 °C, 100 μmol·m^{-2}·s^{-1}, LL). The results showed that the plant height, stem diameter, leaf area, shoot fresh/dry weight, and underground fresh/dry weight of violet inoculated with PGPR were 40%, 17%, 13%, 25%, 41%, 30%, and 38% higher than the control, respectively. In addition, the total root length, mean root diameter, number of root tips, number of bifurcated root tips, and root vigor were 24%, 23%, 14%, 30%, and 21% higher in the inoculated group than in the control group, respectively. PGPR promoted chlorophyll accumulation in plants, and the net photosynthetic rate of violets was 32% higher in plants inoculated with PGPR than in control plants. PGPR also significantly promoted the maximum photochemical conversion efficiency of photosystem II (PSII) and the potential activity of PSII under UV light. After inoculation with PGPR, the content of MDA decreased by 16.8%, and the activities of superoxide dismutase and peroxidase increased by 19.2% and 20.0%, respectively. Therefore, PGPR can promote the growth of violet plants by increasing the photosynthetic capacity, activating the antioxidant enzyme system, and reducing damage induced by exposure to low light intensity.

Keywords: antioxidant enzyme; chlorophyll fluorescence; low temperature and weak light; plant growth-promoting rhizobacteria; photosynthesis

Introduction

Environmental problems are receiving increased attention from researchers and policymakers. Soils can become barren when pesticides and fertilizers are used for long periods, and this can introduce pollutants into the environment (Molina *et al.*, 2020). There is thus a need to increase the use of alternatives to fertilizers to

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promote plant growth, such as innovative technologies (Cordero *et al*., 2018). Rhizosphere growth-promoting bacteria (PGPR) can colonize plant roots and activate soil enzymes. The exudates produced by plant roots can promote the growth of PGBR, enhance the absorption and utilization of mineral nutrients by plants, and even inhibit the growth of harmful microorganisms (Yang *et al*., 2009; Vurukonda *et al*., 2016). These bacteria are referred to as PGPR because they can directly or indirectly promote plant growth. These rhizosphere microorganisms can fix nitrogen, dissolve phosphorus, produce plant growth hormones, function as iron carriers, and induce disease resistance (Deepa *et al*., 2010). PGPR are not pathogenic to plants, and they prevent diseases and improve the resistance of plants to abiotic stress (Toju *et al*., 2018).

The growth and development of plants are affected by many factors, especially temperature and light. Low temperature and low light (LL) stress reduce the electron transfer rate between plant tissues and affect the growth and development of plants (Avenson *et al*., 2005; Mohanty *et al*., 2006). Multiple enzymes are required in the carbon assimilation process. LL can affect the carbon assimilation process by inhibiting enzyme activity, which negatively affects photosynthesis and chlorophyll fluorescence (Ploschuk *et al*., 2014; Murchie *et al*., 2013). The production of O^{2-} and H_2O_2 disrupts active oxygen metabolism, thus intensifying the lipid peroxidation of the cell membrane and damaging the cell membrane of plants (Tjus *et al*., 1998). H_2O_2 can interact with O^{2-} to produce oxygen free radicals, which further damage cells. SOD is one of the most important free radical scavenging enzymes in plant cells. Its main function is to scavenge O^{2-} and generate H_2O_2. POD and CAT can eliminate H_2O_2 in the body, which maintains active oxygen homeostasis in cells and protects the structure of the cell membrane (Asada *et al*., 2006; Huseynova *et al*., 2014; Shafi *et al*., 2015).

Violets (*Matthiola incana*) are a perennial herb native to southern Europe and the Mediterranean coast. Violets prefer regions with mild climates in winter, and their optimal growth temperature ranges from 15 to 18°C. Violets can withstand low temperatures for short periods, but they do not tolerate shade. The growth and development of violets in greenhouses in northern China are often negatively affected by LL in winter. In addition, the production costs of cultivating flowers in northern China in winter are high, and the application of large amounts of chemical fertilizers under greenhouse cultivation can pollute the soil and reduce environmental water quality. Therefore, PGPR could be used as a microbial fertilizer to promote the sustainable development of agriculture (Singh *et al*., 2015). Here, we evaluated the efficacy of applying PGPR as inoculant. Specifically, we investigated the effects of PGPR inoculation on the growth, photosynthetic pigments, photosynthetic properties, and chlorophyll fluorescence characteristics of violet under LL stress. Our findings will aid future studies of the molecular mechanism of chilling and low light stress tolerance in violet leaves and have implications for the breeding of violet plants with LL stress tolerance.

Materials and Methods

Plants and PGPR

The 'Noble' violet variety (purchased from Zhejiang Hongyue Flowers Co., Ltd.) was used in experiments. *Pseudomonas fluorescens* was used as the experimental PGPR (PS2-6). *Pseudomonas fluorescens* was isolated, identified, and preserved from the rhizome of clover and alfalfa. The PGPR strain was cultivated with beef extract peptone medium and inorganic salt medium. Soil that had been sterilized using high temperature and high-pressure steam at 121 °C for 2 h was used in experiments. Violet seeds were soaked in 10% H_2O_2 for 10 min to disinfect the surface; they were then placed on filter paper to dry. The pot containers were disinfected with 75% alcohol solution. The seeds were germinated on moist filter paper saturated with tap water in a climate incubator at a temperature of 25 °C in the dark for 3-4 days; they were then transplanted to the pot (9 × 9 cm) after 26 days (approximately 2-4 true leaves) in the greenhouse. Two seedlings were left in each pot with regular water and fertilizer management under the following conditions: temperature, 28/18 °C (day/night); 12 h/12 h light/dark photoperiod; PPFD, 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$; and relative humidity, 65-70%.

Plants were watered three times a week before budding. According to the fertility level of the culture substrate and plant growth, 200 mL of 30% Hoagland nutrient solution was added in the middle and late stages. Basic physical and chemical properties of soil: pH, 6.86; organic matter content, 5.4 g/kg; total nitrogen, 1.18 g/kg; total phosphorus, 0.4 g/kg; total potassium, 5.6 g/kg; alkali-hydrolyzed nitrogen, 56 mg/kg; available phosphorus, 4.7 mg/kg; and available potassium, 50.2 mg/kg.

Experimental design

The experiment was carried out in the artificial climate room of Qingdao Agricultural University. Seedlings of similar size with four fully expanded leaves were transferred to growth chambers with different temperatures (°C) and light ($\mu mol \cdot m^{-2} \cdot s^{-1}$) intensities: normal temperature and normal light intensity (18 °C/11 °C, 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, NN), low temperature and normal light intensity (4 °C/1 °C, 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, LN), normal temperature and low light intensity (18 °C/11°C, low light intensity (18 °C/11 °C, 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, NL), and low temperature and low light intensity (4 °C/1 °C, 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, LL). The experiment comprised eight treatments: four in which *Pseudomonas fluorescens* Ps2-6 was inoculated (NNP, LNP, NLP, and LLP), and four in which plants were not inoculated (control), including NNC, LNC, NLC, and LLC. Five replications of each treatment were performed. Ten mL of 3×10^8 CFU/mL Ps2-6 were inoculated in the Ps2-6 treatments, and the same amount of sterilized inoculum and inoculum filtrate were added in the control.

Growth parameters

Plant height was determined by measuring the distance between the violet rhizome and the plant growth point with a ruler. The stem diameter was determined by measuring the base diameter of the violet stem with vernier calipers. The surface fresh weight and underground fresh weight were measured using an electronic balance after draining the surface water of violet plants and washing them with clean water. The dry weight of the aboveground and underground parts was measured using an electronic balance after plants were killed at 105 °C for 30 min and then dried until a constant weight was achieved at 80 °C. The blade area was measured by scanning with a Yaxin-1241 portable leaf blade area meter. Root morphological traits were determined using an LA-S series plant root system analyzer.

Activities of antioxidant enzymes

The content of malondialdehyde (MDA) was determined using the thiobarbituric acid (TBA) chromogenic method (Velikova *et al*., 2000), the activity of superoxide dismutase (SOD) was determined using the nitrogen blue tetrazolium (NBT) method (Giannopolitis *et al*., 1977), the activity of peroxidase (POD) was determined using the guaiacol method (Knörzer *et al*., 1996), and the activity of catalase (CAT) was determined using the hydrogen peroxide reduction method (Chance *et al*., 1955).

Photosynthetic parameters

The net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and intercellular CO_2 concentration (Ci) of the fourth fully expanded leaves were measured using a portable photosynthesis system (CIRAS-3, PP-systems, UK) under clear and cloudless weather (9:30-11:30). Data were recorded after the values stabilized.

Content of photosynthetic pigments and fluorescence parameters

The chlorophyll of violet leaves was extracted using 95% ethanol. The chlorophyll fluorescence parameters of leaves were measured using a Pocket PEA fluorescence analyzer under clear and cloudless weather (9:30-11:30). After being stored in the dark at room temperature for 30 min, measurements were taken from three areas on the fourth unfolding leaves. First, the minimal fluorescence (F_0) was recorded after leaves were illuminated with a measuring light; the maximum Chl fluorescence (F_m) was obtained after a saturation pulse,

the PS Ⅱ potential activity was defined as F_v/F_0, and the maximum photochemical efficiency was calculated as F_v/F_m.

Statistical analysis

Three replications of each experiment were performed. The data were processed and plotted using Microsoft Excel 2016 software. All statistical analyses were conducted using SPSS 11.5. Analysis of variance (ANOVA) was used to evaluate the effects of treatments on variables ($p < 0.05$). Significant differences between individual means were determined using Duncan's multiple range tests ($p < 0.05$).

Results

Plant growth

NL, LN, and LL significant inhibited violet growth. Compared with the NNC group, the plant height, stem diameter, leaf area, aboveground fresh weight, belowground fresh weight, aboveground dry weight, and belowground dry weight of violet in the LLC group were decreased by 36%, 33%, 39%, 26%, 63%, 45%, and 70%, respectively. Inoculation of PGPR can alleviate the inhibitory effect of LL. Compared with LLC, plant height, stem diameter, leaf area, fresh weight of aerial part and underground part of fresh weight, dry weight of above ground and underground part of the dry weight of PGPR inoculation group (LLP) increased by 40%, 17%, 13%, 25%, 41%, 30% and 38%, respectively. In addition, the height, stem diameter, leaf area, aboveground fresh weight, bellow fresh weight, aboveground dry weight, and bellow dry weight of violet in NLP group were increased by 6%, 6%, 6%, 18%, 45%, 7%, and 21% compared with NLC, respectively. Under LN condition, violet plant height, stem diameter, leaf area, aboveground fresh weight, underground fresh weight, aboveground dry weight and underground dry weight of LNP increased by 9%, 8%, 24%, 26%, 35%, 16% and 17% respectively compared with LNC (Table 1).

Root system architecture and root activity

NL, LN, and LL inhibited root architecture and root activity of violet to different extents. Compared with the NNC group, the total root length, average root diameter, number of root tips, number of bifurcated root tips, and root activity of violet in the LLC group decreased by 45%, 37%, 56%, 52%, and 53%, respectively. Inoculation with PGPR, however, significantly improved the root parameters of violet under LL. Compared with LLC, the total root length, average root diameter, number of root tips, number of bifurcated root tips, and root activity of LLP increased by 24%, 23%, 14%, 30%, and 21%, respectively. In addition, total root length, mean root diameter, number of root tips, number of bifurcated root tips, and root vigor of violet in the NLP group increased by 14%, 15%, 6%, 27%, and 24%, respectively, compared with the NLC group. However, the total root length, average root diameter, number of root tips, number of bifurcated root tips and root activity of violet in the LNP group increased by 7%, 6%, 6%, 22% and 21%, respectively (Table 2).

Table 1. Effect of low temperature and low light on the growth index of violet

Treatment	Plant height (cm)	Stem diameter (cm)	Leaf area (cm^2)	Fresh weight of aboveground part (g)	Fresh weight of underground part (g)	Dry weight of aboveground part (g)	Dry weight of underground part (g)
NNC	15.87±0.32ab	0.45±0.02a	1136.40±23.61b	13.23±0.26cd	2.68±0.12b	1.37±0.04b	0.27±0.02b
NLC	15.10±0.21cd	0.36±0.02bc	928.23±21.93c	11.33±0.38e	1.52±0.18ef	0.98±0.03d	0.14±0.01de
LNC	14.23±0.38d	0.37±0.01bc	771.00±21.13de	11.30±0.29e	1.82±0.11de	1.04±0.04cd	0.18±0.02cd
LLC	10.20±0.32e	0.30±0.01d	689.00±8.33e	9.83±0.24f	0.98±0.06g	0.76±0.06e	0.08±0.01f
NNP	16.43±0.15a	0.49±0.01a	1405.83±48.35a	15.47±0.48a	3.74±0.26a	2.02±0.11a	0.47±0.01a
NLP	16.07±0.15ab	0.38±0.01bc	986.67±39.37c	13.37±0.13bc	2.20±0.08cd	1.05±0.06cd	0.17±0.02cd
LNP	15.53±0.25bc	0.40±0.01b	955.27±29.95c	14.23±0.18b	2.46±0.04bc	1.21±0.06bc	0.21±0.01c
LLP	14.33±0.29d	0.35±0.01c	779.67±14.91d	12.27±0.45de	1.38±0.01f	0.99±0.03d	0.11±0.01ef
Test of significance							
PGPR	*	NS	*	**	*	*	*
Low light intensity	*	*	*	*	**	**	**
Low temperature	**	*	**	*	**	*	*
Low temperature × Low light intensity	**	**	**	**	**	**	**
PGPR × Low light intensity	NS	*	*	*	*	*	*
PGPR × Low temperature	*	*	*	*	*	*	*
PGPR× Low temperature × Low light intensity	**	**	**	*	**	**	**

Note: The data are shown as the mean ± standard deviation of three replicates. Different lowercase letters (a, b, c, and d) indicate significant differences among treatments. The significance of differences was determined using Duncan's multiple range tests, and the threshold for statistical significance was $p < 0.05$. NN: normal temperature and normal light intensity (18 °C/11 °C, 500 μmol·m^{-2}·s^{-1}); NL: normal temperature and low light intensity (18 °C/11 °C, 100 μmol·m^{-2}·s^{-1}); LN: low temperature and normal light intensity (4 °C/1°C, 500 μmol·m^{-2}·s^{-1}); LL: low temperature and low light intensity (4 °C/1 °C, 100 μmol·m^{-2}·s^{-1}); *Pseudomonas fluorescens* (PS2-6) inoculation group (NNP, LNP, NLP, and LLP), and non-inoculation groups including NNC, LNC, NLC, and LLC; "NS" indicates that the differences are not significant, * $p < 0.05$, ** $p < 0.01$.

Table 2. Effects of low temperature and low light on the root configuration and root activity of violet

Treatment	Total root length (cm^2)	Average diameter (mm)	Root tip number	Number of root tips bifurcating	Root activity (μg·g^{-1}·h^{-1})
NNC	398.38±10.15b	0.35±0.01b	1610.33±76.84b	3342.67±61.95b	119.86±4.49b
NLC	296.86±5.45e	0.27±0.01d	862.33±20.93de	2202.67±47.26e	77.17±4.13ef
LNC	364.37±2.92c	0.31±0.02c	944.00±4.93cd	2475.00±78.9d	86.16±2.71de
LLC	219.49±8.09f	0.22±0.01e	710.33±1.76f	1590.33±23.26f	56.90±2.68g
NNP	624.62±8.84a	0.39±0.01a	1976.33±85.27a	3727.67±343.83a	148.61±6.99a
NLP	337.87±13.72d	0.31±0.01c	915.33±11.11cde	2792.00±155.62c	95.40±1.64cd
LNP	390.54±4.29b	0.33±0.01bc	1000.00±8.72c	3023.33±292.34c	103.85±1.02c
LLP	272.76±13.33e	0.27±0.01d	808.00±9.17ef	2070.67±175.11e	69.00±1.55f
Test of significance					
PGPR	*	*	*	*	*
Low light intensity	**	**	**	**	**
Low temperature	*	*	*	**	**
Low temperature ×Low light intensity	**	**	**	**	*
PGPR× Low light intensity	*	*	*	*	*

PGPR× Low temperature	NS	NS	*	*	*
PGPR× Low temperature × Low light intensity	**	**	**	*	**

Note: The data are shown as the mean ± standard deviation of three replicates. Different lowercase letters (a, b, c, and d) indicate significant differences among treatments. The significance of differences was determined using Duncan's multiple range tests, and the threshold for statistical significance was $p < 0.05$. NN: normal temperature and normal light intensity (18 °C/11 °C, 500 μmol·m-2·s-1); NL: normal temperature and low light intensity (18 °C/11 °C, 100 μmol·m-2·s-1); LN: low temperature and normal light intensity (4 °C/1°C, 500 μmol·m-2·s-1); LL: low temperature and low light intensity (4 °C/1 °C, 100 μmol·m-2·s-1); Pseudomonas fluorescens (PS2-6) inoculation group (NNP, LNP, NLP, and LLP), and non-inoculation groups including NNC, LNC, NLC, and LLC; "NS" indicates that the differences are not significant, * $p < 0.05$, ** $p < 0.01$.

Chlorophyll content

Chlorophyll content of violet in LL group was significantly lower than that in NN group. LL, under the condition of inoculation PGPR (LLP), violet chlorophyll content was significantly increased by 22%. The chlorophyll content of violet in the NLP group was 13% higher than that in the NLC group, while that in the LNP group was 9% higher than that in the LNC group, but the effect was not significant (Figure 1).

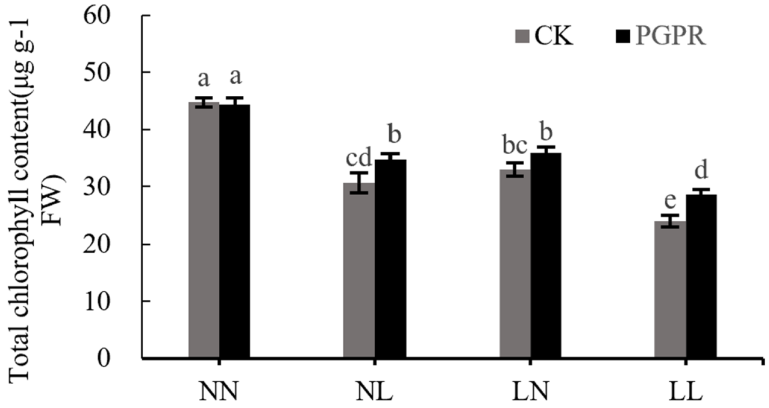

Figure 1. Effect of inoculation of PGPR on the total chlorophyll content of violet under low temperature and low light stress

NN: normal temperature and normal light intensity (18 °C/11 °C, 500 μmol·m^{-2}·s^{-1}); NL: normal temperature and low light intensity (18 °C/11 °C, 100 μmol·m^{-2}·s^{-1}); LN: low temperature and normal light intensity (4 °C/1 °C, 500 μmol·m^{-2}·s^{-1}); LL: low temperature and low light intensity (4 °C/1°C, 100 μmol·m^{-2}·s^{-1}).

Gas exchange

NL, LN and LL conditions have significant effects on the gas exchange parameter of violet, LLC and NNC group, compared the violet leaf net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), water use efficiency (WUE) was reduced by 52%, 48%, 71% and 57% respectively, intercellular CO_2 concentration (Ci) increased by 80%. PGPR inoculation significantly promoted Pn, Gs, Ci, Tr, and WUE of violets under LL. Under the condition of LL, Pn, Gs, Tr, and WUE of PGPR (LLP) violet were increased by 46%, 38%, 30%, and 34%, respectively, compared with LLC, while Ci was decreased by 21%. In addition, under the NL condition, the violet Pn, Gs, Tr, and WUE of NLP increased by 5%, 11%, 15%, and 18%, respectively, compared with NLC, while Ci decreased by 19%. The Pn, Gs, Tr, and WUE of violets in LNP increased by 13%, 17%, 8%, and 27%, respectively, compared with those in LNC, while Ci decreased by 23% (Table 3).

Table 3. Effect of inoculation of PGPR on the gas exchange parameters of violet under low temperature and low light conditions

Treatment	Pn ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Gs ($mmol \cdot m^{-2} \cdot s^{-1}$)	Ci ($\mu mol \cdot m^{-1}$)	Tr ($g \cdot m^{-2} \cdot h^{-1}$)	WUE ($\mu mol \cdot m^{-2} \cdot s^{-1}$)
NNC	2.70±0.12b	80±3.78b	233.67±5.61e	2.7±0.1b	3.43±0.09b
NLC	1.83±0.09d	62.67±2.33de	362±16.17bc	2±0.06c	2.2±0.17cde
LNC	2.13±0.08cd	65±2.52d	378±10.69b	1.97±0.23c	2.13±0.09de
LLC	1.30±0.12e	41.67±1.45f	419.67±10.17a	0.77±0.09d	1.47±0.15f
NNP	3.30±0.12a	90±1.53a	270±13.89d	3.4±0.12a	4.6±0.35a
NLP	1.93±0.07d	69.67±2.91cd	292.33±18d	2.3±0.12c	2.6±0.15cd
LNP	2.40±0.10bc	76.33±1.76bc	291.67±7.89d	2.12±0.09c	2.7±0.17c
LLP	1.90±0.12d	57.67±1.45e	329.67±7.84c	1±0.06d	1.97±0.12ef
Test of significance					
PGPR	*	*	*	*	*
Low light intensity	**	**	**	*	*
Low temperature	*	**	**	*	**
Low temperature × Low light intensity	**	**	**	**	**
PGPR × Low light intensity	**	*	**	*	*
PGPR × Low temperature	NS	NS	**	*	*
PGPR × Low temperature × Low light intensity	**	**	**	**	**

Note: The data are shown as the mean ± standard deviation of three replicates. Different lowercase letters (a, b, c, and d) indicate significant differences among treatments. The significance of differences was determined using Duncan's multiple range tests, and the threshold for statistical significance was $p < 0.05$. NN: normal temperature and normal light intensity (18 °C/11 °C, 500 μmol·m-2·s-1); NL: normal temperature and low light intensity (18 °C/11 °C, 100 μmol·m-2·s-1); LN: low temperature and normal light intensity (4 °C/1°C, 500 μmol·m-2·s-1); LL: low temperature and low light intensity (4 °C/1 °C, 100 μmol·m-2·s-1); Pseudomonas fluorescens (PS2-6) inoculation group (NNP, LNP, NLP, and LLP), and non-inoculation groups including NNC, LNC, NLC, and LLC; "NS" indicates that the differences are not significant, * $p < 0.05$, ** $p < 0.01$.

Chlorophyll fluorescence

Under LL stress, the F_v/F_m and F_v/F_0 of violet decreased, and the effect of low light stress was more significant. The effect of LL on the maximum photochemical efficiency of PSII in violet was alleviated by PGPR inoculation, and the potential activity of PSII was significantly increased following PGPR inoculation. The Fv/Fm and Fv/F_0 of LLP violet increased by 25% and 27%, respectively, compared to LLC. In NL, the Fv/Fm and Fv/F0 of violines inoculated with PGPR treatment (NLP) increased by 15% and 6%, respectively, while in LN, the Fv/Fm and Fv/F_0 of violines inoculated with PGPR (LNP) had no significant effect (Figure 2, Table 4).

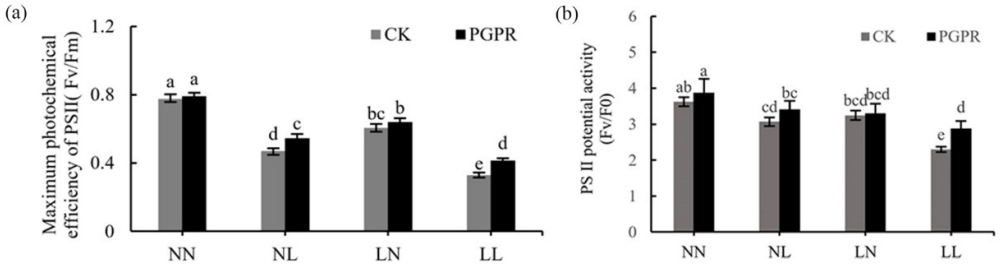

Figure 2. Effect of PGPR inoculation on chlorophyll fluorescence parameters in violet. (a) the maximum photochemical efficiency of PSII (F_v/F_m), (b) the PSII potential activity (F_v/F_0)

Table 4. Effects of PGPR on violet fluorescence parameters under low temperature and low light stress

Source of variation	Total chlorophyll	Maximum photochemical efficiency of PSII (F_v/F_m)	Potential activity of PSII (F_v/F_0)
PGPR	NS	NS	*
Low light intensity	**	**	*
Low temperature	**	*	*
Low temperature × Low light intensity	**	**	**
PGPR × Low light intensity	*	**	*
PGPR × Low temperature	*	*	*
PGPR × Low temperature × Low light intensity	**	**	**

Membrane permeability and antioxidant enzyme activity

Under NL, LN and LL conditions, the activities of antioxidant enzymes were significantly decreased, while MDA content was significantly increased. PGPR inoculation significantly increased the activities of antioxidant enzymes and MDA content in violet. Compared with the NNC group, the MDA content in the LLC group was significantly increased by 102%, while SOD, POD and CAT were decreased by 43%, 56% and 43%, respectively. Compared with the NNC group, the MDA content of violet in the NLC group increased by 53%, and SOD, POD, and CAT decreased by 21%, 27%, and 46%, respectively. Compared with the NNC group, the MDA content of violets in the LNC group increased by 75%, and SOD, POD, and CAT decreased by 6%, 18%, and 18%, respectively.

PGPR inoculation had a significant effect on the membrane permeability and antioxidant enzyme activity of violet cells under NL, LN, and LL conditions. The contents of MDA in violaceous of LLP decreased by 17% compared with LLC, while SOD, POD and CAT increased by 19%, 20% and 15%, respectively. In addition, under NL conditions, the content of MDA in violats inoculated with PGPR (NLP) decreased by 14%, while SOD, POD, and CAT increased by 1%, 4%, and 18%, respectively. Under the condition of LN, vaccination PGPR (LNP) violet the content of MDA was reduced by 20%, SOD, POD, CAT, respectively increased by 10%, 38% and 24%, respectively (Table 5).

Table 5. Effects of low temperature and low light stress on membrane permeability and the antioxidant enzyme activity of violet

Treatments	MDA (μmol·g^{-1})	SOD (U/g·min)	POD (U/g·min)	CAT (U/g·min)
NNC	17.63±0.93g	135.32±3.90 bc	163.97±5.78b	3.96±0.14b
NLC	27.04±0.86cd	107.01±1.88d	120.43±2.15d	2.12±0.26d
LNC	30.85±1.27b	127.14±4.00c	133.8±3.01cd	3.26±0.17c
LLC	35.54±0.91a	76.78±1.86 f	72.33±8.92e	2.25±0.21d
NNP	20.71±0.39f	152.74±3.80a	194.97±3.98a	4.53±0.18a
NLP	23.23±0.65ef	108.11±2.15d	124.93±5.43d	2.51±0.13d
LNP	24.57±0.56de	139.44±3.56b	184.47±3.21c	4.04±0.24ab
LLP	29.58±0.92bc	91.53±3.56e	86.80±4.52e	2.58±0.13d
Test of significance				
PGPR	*	*	*	*
Low light intensity	**	*	**	**
Low temperature	**	*	*	*
Low temperature × Low light intensity	**	**	*	**
PGPR × Low light intensity	*	*	**	**
PGPR × Low temperature	**	*	*	*
PGPR × Low temperature × Low light intensity	**	**	**	**

Discussion

PGPR can promote plant growth through their effects on plant roots (Vacheron *et al*., 2013). The growth rate and dry matter content of plants are reduced under LL stress (Jampeetong *et al*., 2020; Chen *et al*., 2020). Previous studies have shown that inoculation with PGPR can promote plant growth and alleviate damage caused by LL (Singh *et al*., 2017; Ai *et al*., 2017). Under low temperature or low light stress, the effect of PGPR on plant growth is weakened, and these findings are consistent with the results of several studies of plants (Damodarachari *et al*., 2018; Su *et al*., 2015; Paulucci *et al*., 2015). In this study, we found that increases in dry matter accumulation in plants under normal temperature and light conditions might stem from increases in the carbon accumulation and CO_2 assimilation rate given that PGPR promote photosynthesis in violets. Under LL stress, PGPR might promote the accumulation of photosynthetic products in the roots, thus enhancing the uptake of mineral elements by plant roots and the growth of the aboveground and underground parts.

Photosynthesis is one of the most basic processes mediating the circulation of materials in plants. It can convert light energy into chemical energy, which is required by plants for growth and development (Pan *et al*., 2020). Chlorophyll is mainly used to absorb and transmit light energy. Changes in the content of total chlorophyll (Chl) induce changes in the photosynthetic rate (Singh *et al*., 2018). Previous studies have shown that the content of Chl a and Chl b in Aquilaria sinensis decreases significantly under LL stress, and this results in the inhibition of photosynthesis (Yuan *et al*., 2013). The Pn, Gs, and Tr of pepper (*Capsicum annuum*) seedlings decrease and Ci increases under LL stress (Yang *et al*., 2009). The decrease in Gs and Ci in plants indicates that the assimilation of CO_2 is limited by stomatal factors under stress. Non-stomatal factors also

result in decreases in Gs and increases in Ci (Yu *et al*., 2013). In this study, low temperature and weak light inhibited chlorophyll synthesis, which led to a decline in the photosynthetic rate and the CO_2 utilization rate; this resulted in the continuous accumulation of CO_2 between cells. PGPR alleviated the inhibition of LL stress on the photosynthesis of violet, which decreased the effect of non-stomatal limiting factors on Gs and improved the shade tolerance and cold tolerance of violet.

The PSII complex plays an important role in plant leaf photosynthesis and adaptation to environmental stress (Allen *et al*., 1981). Chlorophyll fluorescence parameters can reflect the effects of adverse stress conditions on photosynthesis, as well as the efficiency of plant light energy absorption, transformation, and transmission (Begcy *et al*., 2012). Previous studies have shown that LL induce decreases in the total chlorophyll content of tomato (*Solanum lycopersicum*) and the maximum photochemical efficiency of PSII (F_v/F_m) and damage to the PSII complex (Fang *et al*., 2018). The chlorophyll fluorescence parameters F_v/F_m and F_v/F_0 of violet in this experiment were reduced under LL stress, and this is consistent with the results of previous studies (Pradhan *et al*., 2019; AL-Shoaibi *et al*., 2008). We speculate that LL conditions damaged the photochemical reaction center of PSII in leaves, which weakens the ability of violet to capture light and utilize light energy. Both F_v/F_m and F_v/F_0 of the violets inoculated with PGPR were higher than those of non-inoculated plants under normal light conditions/normal temperature or low temperature/low light conditions, indicating that PGPR could protect the photosynthetic system from the deleterious effects of stress and improve the electron conversion efficiency of PSII. PGPR enhanced the ability of the photosynthetic reaction center to capture photons and use light energy, improved the photosynthetic efficiency of violet, and alleviated the inhibitory effects of LL stress.

Chloroplasts, mitochondria, and peroxisomes are the main organelles that produce ROS during aerobic metabolic processes, such as photosynthesis and respiration, in plants. Plants can also produce ROS in the plasma membrane and exophytes in response to various stress signals (Lee *et al*., 2018). Cell membranes are important for regulating the transport and exchange of substances in plants (Mongrand *et al*., 2010). Plants produce more ROS under stress conditions (Qi *et al*., 2018). The accumulation of ROS can cause membrane lipid peroxidation, and MDA is one of the most important substances produced in this process. MDA is thus considered an indicator of the degree of membrane lipid peroxidation and can reflect the degree of damage to plants under adverse conditions (Kanazawa *et al*., 2000). SOD, POD, CAT, and other key enzymes and osmotic regulators play a key role in ensuring the integrity of the plasma membrane and resistance to damage to membrane lipids caused by ROS (You *et al*., 2015). A previous study has shown that the SOD, POD, and CAT activities of cucumber decrease under low temperature stress (Li *et al*., 2017). In addition, Arbuscular mycorrhizal fungi alleviated reactive oxygen species damage by elevating the SOD, POD, and CAT activities of snapdragon (Li *et al*., 2024). These findings indicate that plants can alter the activities of these enzymes or the amount of antioxidant substances to eliminate the excessive accumulation of ROS in plants. The results of our study showed that SOD and POD activities were significantly higher and the MDA content was lower in violet plants inoculated with PGPR than in non-inoculated plants under LL stress; PGPR inoculation had no significant effect on CAT activity. This might be explained by increases in the concentration of intracellular ROS under LL stress. The accumulation of ROS can destroy the structure of antioxidant enzymes or reduce their activity. PGPR can mitigate the lipid peroxidation of the cell membrane and apoptosis.

Conclusions

LL stress inhibited the growth and development of violet, but after inoculation with PGPR, the damage induced to plants by LL stress was significantly reduced. The results of this study indicated that PGPR inoculation had a positive effect on the growth and physiological metabolism of violet under LL stress. To clarify the specific mechanism underlying the positive effects of PGPR, additional studies of the effects of

PGPR on stress response genes and metabolites in violet are needed. Determining the importance of other factors, such as plant root exudates, the composition of soil microbes, and soil physical and chemical properties, will be key for understanding how PGPR promote the growth of plants.

Authors' Contributions

W.L. and S.G. designed the experiment. Y.Z. and Y.S. performed the experiment. W.L. participated in writing the manuscript. W.L. participated in revising the manuscript.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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