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Exogenous paclobutrazol can relieve the low irradiance stress in *Capsicum annuum* seedlings

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Abstract

Irradiance is one of the main limiting factors affecting the production of pepper (*Capsicum annuum* L.) in facility production. It is therefore important to measure the growth of pepper seedlings under low irradiance and to understand how to relieve low radiation stress. In this study, pepper seedlings were cultivated under low irradiance and were treated with gibberellic acid (GA_3) and paclobutrazol (PP333). Agronomic and physiological characteristics of the pepper seedlings were analyzed. Under low irradiance, the plant height, leaf area, and the chlorophyll (Chl) and malondialdehyde (MDA) content of pepper seedlings were higher than under normal irradiance, while the content of proline, and soluble protein and Chl *a*/*b* ratio of pepper seedlings were lower than those under normal irradiance; exogenous GA_3 had a similar effect. When PP333 was applied to the seedlings, abnormal growth was mitigated. The effects of exogenous GA_3 and PP333 on Chl fluorescence parameters were also analyzed. Under low irradiance, the maximum quantum yield reduction of photosystem (PS II) (F_v/F_m) increased significantly, and the reaction center initiated the corresponding defense mechanism for timely dissipation of excess excitation energy to reduce the damage to the plant. These results showed that spraying gibberellin under normal irradiance conditions had similar effects on pepper seedlings as under low irradiance conditions. Exogenous PP333 relieved the growth reduction by increasing the content of Chl and soluble protein and enhancing some photosynthetic parameters. These results suggest that exogenous PP333 can alleviate the abnormal growth of pepper under low radiation stress.

Keywords: *Capsicum annuum*, chlorophyll content, chlorophyll fluorescence, GA_3, growth, paclobutrazol, pepper, proline.

Introduction

Radiation, one of the most important environmental factors, is essential for plant growth and development (Li *et al*. 2017). Morphological and physiological adaptations in plants can be mediated through morphogenetic responses and radiation dependent adjustments in photosynthesis (Hoffmann *et al*. 2016). Radiation is considered one of the

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Abbreviations: ABS/CS_o - absorption flux per cross section (CS) (at t = 0); ABS/RC - radiation energy absorbed by RC; Chl - chlorophyll; DI_o/CS_o - dissipated energy flux per cross section (CS) (at t = 0); DI_o/RC - light energy dissipated by RC (at t = 0); ET_o/CS_o - electron transport flux per cross section (CS) (at t = 0); ET_o/RC - the RC unit of the reaction center captures the energy used for electron transport (at t = 0); F_m - maximal chlorophyll fluorescence measured in the dark-adapted state during the application of a saturating radiation pulse; F_o - minimal chlorophyll fluorescence measured in the dark-adapted state when all PS II RCs are open; F_v/F_m - maximum quantum yield of PS II photochemistry measured in the dark-adapted state; F_v/F_0 - efficiency of the water-splitting complex on the donor side of PS II; GA_3 - gibberellic acid; MDA - malondialdehyde; P_N - net photosynthetic rate; PP333 - paclobutrazol; PS - photosystem; LCP - light compensation point; RC - reaction center of PS II; TBA - thiobarbituric acid; TCA - trichloroacetic acid; TR_o/CS_o - trapped energy flux per cross section (CS) (at t = 0); TR_o/RC - RC captures energy used to restore QA (at t = 0).
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major environmental factors controlling leaf morphology (Chai *et al*. 2018). Pepper is sensitive to the change in irradiance, and shading may increase the yield, but improper shading may lead to its reduction (Chen *et al.* 1998). In order to make more use of the limited radiation energy, hot (sweet) pepper seedlings expand the area of the photosynthetic organs under lower irradiance. However, proper shading can increase the fruit mass, yield, net photosynthetic rate (P_N), and light compensation point (LCP) of pepper (Sui *et al*. 2006). Under low irradiance, P_N, dark respiration rate, transpiration rate, water use efficiency, LCP, carbon dioxide compensation point, and carboxylation efficiency of pepper seedlings decreased, while the height/stem diameter ratio and specific leaf area increased. Under low irradiance, the formation of biological and economic yield was significantly inhibited, and the distribution of assimilates to the stem and leaf increased, while the distribution to fruit decreased (Ni *et al*. 2020). Hot pepper under weak irradiance had lower LCP, higher relative healthy index, higher relative yield per plant, and higher relative setting rate, all which shows a stronger capability of weak irradiance tolerance than sweet pepper (Sui *et al*. 2006).

Gibberelic acid promotes physiological changes that regulate plant growth and development patterns (Falcioni *et al*. 2018). GA_3, a plant growth regulator, has been widely used in horticulture and agricultural production (Erin *et al*. 2008). GA_3 can break seed dormancy and promote germination, plant flowering, and improve yield (Sandoval-Oliveros *et al*. 2017). The effects of GA_3 vary among plant species and cultivars. Relevant studies showed that exogenous GA_3 could induce the early development of buds and make peony blossom in autumn. GA_3 can also promote internode elongation, the formation of male flowers in cucumbers, induce grapes to produce seedless fruit, make banana maintain a higher quality and its delay aging in storage.

As an inhibitor of endogenous GAs synthesis, PP333 was developed in the 1980s (Hedden *et al*. 1986). The biosynthesis of GAs can be separated into three stages: terpene cyclases acting in proplastids, monooxygenases associated with the endoplasmatic reticulum, and dioxygenases located in the cytosol. PP333 blocks cytochrome P450-dependent monooxygenases, thereby inhibiting oxidation of *ent*-kaurene into *ent*-kaurenoic acid (Rademacher *et al*. 2000). In agriculture, PP333 is mainly used to delay the growth of plants, inhibit plant elongation, increase the resistance of plants, and improve yield. It was reported that PP333 plays multiple roles on plant protection to resist environmental pressures, such as drought and salt exposure (Percival *et al*. 2008). The results showed that PP333 significantly ameliorated the adverse effects of NaCl stress in *Vigna unguiculata* plants (Manivannan *et al*. 2007). Leaf or substrate spraying can effectively reduce plant height and the MDA content, increase the thickness of the main stem, and increase the leaf Chl content and soluble sugar content (Tekalign *et al.* 2004, França *et al.* 2018, Fan *et al.* 2020, Liu *et al*. 2020).

Pepper and other *Capsicum* species are annual or limited perennial herbaceous plants. As a thermophilic vegetable, pepper is one of the main vegetables cultivated in facilities in China. Irradiance plays an important role in the process of plant growth and development. Low irradiance is considered to be the main limiting factor affecting pepper production in facility cultivation in the Yangtze-Huai River basin in China (Sui *et al*. 2012). It is important to study the influence of low irradiance on pepper. Pepper seedlings sprayed with GA_3 under normal irradiance showed similar phenomena to those sprayed with distilled water under low irradiance. Pepper seedlings were abnormal elongation phenomenon. Exogenous spraying of PP333 can alleviate this phenomenon and ensure the normal growth of plants. Therefore, this paper studies that exogenous spraying of PP333 can reduce abnormal stem elongation of pepper seedlings under low irradiance.

Materials and methods

Plants and experimental design: In the present study, pepper (*Capsicum annuum* L.) cv. Wanfeng Big Bell was used. On September 15, 2019, completely filled pepper seeds of uniform size were selected and planted in the glass greenhouse of the production base in Anhui Agricultural University under the following conditions: a day/night temperatures of 27/12 °C, a 12-h photoperiod, a photosynthetic photon flux density (PPFD) of 320 ± 20 µmol m^{-2} s^{-1}, and a relative humidity of 70 %. Fifteen days after emergence, pepper seedlings with consistent growth (plant height = 7.76 ± 0.5 cm) were selected and divided into L and N growth chambers. Growth chamber N was exposed to natural irradiance of 320 ± 20 µmol m^{-2} s^{-1}; growth chamber L was exposed to low irradiance of 100 ± 20 µmol m^{-2} s^{-1} (Zhang *et al.* 2020). There were six treatments as follows: (1 - 3) under normal irradiance, leaves were sprayed with distilled water (N+CK), 100 mg dm^{-3} GA_3 (N+GA_3), 50 mg dm^{-3} PP333 (N+PP333); (4 - 6) under low irradiance, leaves were sprayed with distilled water (L+CK), 100 mg dm^{-3} GA_3 (L+GA_3), 50 mg dm^{-3} PP333 (L+PP333). All seedlings were exposed to an average relative humidity of 70 %, a 12-h photoperiod, a temperature of 27/12 °C (day/night), and the same water and fertilizer management. Every 4 d, foliar spray treatment was applied for a total of four times; the spray amount per plant was the same, approximately 10 cm^3. The day after treatment, pepper seedlings were measured for morphological indicators, and the pepper leaves were sampled from three independent plants randomly. Measurements and samples were also collected on the day before the first spray treatment. GA_3 (0.1 g) was accurately weighed, dissolved in a small amount of 95 % (v/v) ethanol, and then diluted to 1 dm^3 with distilled water to gain a gibberellin solution with a concentration of 100 mg dm^{-3}. Paclobutrazol (0.05 g) was accurately weighed, dissolved and diluted to 1 dm^3 with methanol solution, and then a 50 mg dm^{-3} PP333 solution was obtained. Both the GA_3 solution and PP333 solution were prepared and used immediately. In this experiment, the results collected after the third spray treatment application were statistically analyzed.

Determination of growth indicators: Plant height was measured from the stem base to the shoot tip, stem thickness was measured at the stem base using a Vernier caliper, and a leaf area meter was used to estimate leaf area. The procedure for measuring leaf thickness was as follows: selecting 10 leaves with the same growth trend, stacking them neatly together, and measuring the thickness of the 10 leaves with a Vernier caliper, so as to obtain the thickness of one leaf. Six plants were selected for each treatment, and each plant was measured three times.

Determination of the chlorophyll content: The Chl content was determined using 50 - 200 mg of the fresh and healthy leaves of pepper, with three replications per treatment. Chl was extracted with 10 cm^3 of 80 % (v/v) chilled acetone, and the absorbances were measured at 645 and 663 nm using a spectrophotometer (*TU1950*, *PERSEE*, Beijing, China) (Zhang *et al*. 2015). The total Chl content (mg g^{-1}(f.m.)) was calculated using a published formula of Arnon *et al*. (1949).

Chlorophyll fluorescence measurements: The rapid Chl fluorescence was measured with a *Han-PEA* continuous excitation fluorometer (*Pocket PEA*, *Hansatech Instruments Ltd., Norfolk, UK)* (Joshi *et al*. 2019). The measurements were collected under clear sky conditions, using fully expanded mature leaves to represent the current growth conditions of the pepper.

Determination of the malondialdehyde content: The MDA content was determined by the thiobarbituric acid (TBA) method (Velikova *et al*. 2000). First, 0.5 g of the leaves was weighed, and 5 cm^3 of 10 % (m/v) trichloroacetic acid (TCA) was added to homogenate samples in an ice bath. Samples were centrifuged at 10 000 *g* for 10 min, and the supernatant was taken. Then, 2 cm^3 of 0.67 % (m/v) TBA was added, the mixture was boiled in a boiling water bath for 30 min, cooled to room temperature, and then centrifuged once more. The supernatant was measured for absorbance values at 450, 532, and 600 nm, using a spectrophotometer, and the control tube was filled with 2 cm^3 of distilled water.

Measurement of soluble protein: The soluble protein content was determined using 0.25 - 0.5 g of the fresh and healthy leaves of pepper, with three replications per treatment. The leaves were ground with 5 cm^3 of distilled water, centrifuged at 766 g for 10 min, and the sediment was discarded. Then, 1 cm^3 of the extract was placed in a test tube, 5 cm^3 of Coomassie Bright Blue reagent was added, shaken well, and the colour was developed for 2 min, after which the absorbance was measured at 595 nm using a spectrophotometer (Sedmak *et al*. 1977).

Measurement of the proline content: The ninhydrin procedure outlined by Bates *et al*. (1973) was followed for analysis of the proline content. A fresh leaf sample (0.5 g) was homogenized in 10 cm^3 of aqueous sulfosalicylic acid (3 %; m/v) and then the homogenized solution was filtered. A solution of 2 cm^3 of acid ninhydrin and 2 cm^3 of glacial acetic acid was added to 2 cm^3 of the filtrate. All samples were placed in a water bath at 80 °C for 1 h, and then they were placed in an ice bath to terminate the reaction. Following the addition of toluene (4 cm^3), the mixtures were mixed for 15 - 20 s using a test tube mixer. The absorbance values were measured at 520 nm (Kaya *et al*. 2019).

Data analysis: Analysis of variance was performed using *IBM SPSS Statistics v. 26*. Significant differences among different treatments were reported at $P < 0.05$, if not indicated otherwise. *GraphPad Prism* software (*GraphPad*, San Diego, CA, USA) was used for diagraph analysis.

Results

The pepper seedlings sprayed with GA_3 under low irradiance were the tallest and had the largest leaf area. The seedling height and leaf area of pepper sprayed with PP333 under normal irradiance were the lowest. Under normal irradiance and low irradiance, the plant height and leaf area of pepper seedlings treated with GA_3 were significantly higher than those treated with distilled water, while those treated with PP333 were lower than those treated with distilled water. (Fig. 1*A,C*). The pepper seedlings sprayed with PP333 under normal irradiance had the thickest leaves. The pepper seedlings sprayed with GA_3 under low irradiance had the smallest stem diameter and the lowest leaf thickness. The stem diameter of pepper seedlings under normal irradiance was significantly higher than that under low irradiance (Fig. 1*B,D*). The stem diameter and leaf thickness of pepper seedlings sprayed with PP333 increased by 18 and 23 % under low irradiance, respectively. Pepper seedlings sprayed with PP333 under low irradiance are similar to sprayed with distilled water under normal irradiance (Fig. 2).

The content of Chl *a* and *b* was the highest when PP333 was sprayed under low irradiance, while the content of Chl *a* and *b* was the lowest when GA_3 was sprayed under normal irradiance. The Chl *a* and Chl *b* content of pepper seedlings under low irradiance was significantly higher than that under normal irradiance, thus low irradiance increased the Chl content in pepper seedlings. PP333 promoted the increase in Chl *a* and Chl *b* content in pepper seedlings under normal irradiance or low irradiance, while GA_3 reduced the content of Chl *a* and Chl *b* in pepper seedlings. Compared with normal irradiance, low irradiance significantly reduced the Chl *a*/*b* ratio. Under the same treatment, GA_3 significantly reduced the Chl *a*/*b* ratio, while PP333 had no significant effect on Chl *a*/*b* ratio (Fig. 3).

Both GA_3 and PP333 were sprayed under normal irradiance, and the effect of them on F_0 was not obvious under low irradiance. Low irradiance resulted in a significant increase in F_m. Under low irradiance, F_m values have no significant difference between GA_3 and PP333 treatment. Under normal irradiance, F_m value significantly increased under PP333 treatment. The effect of low

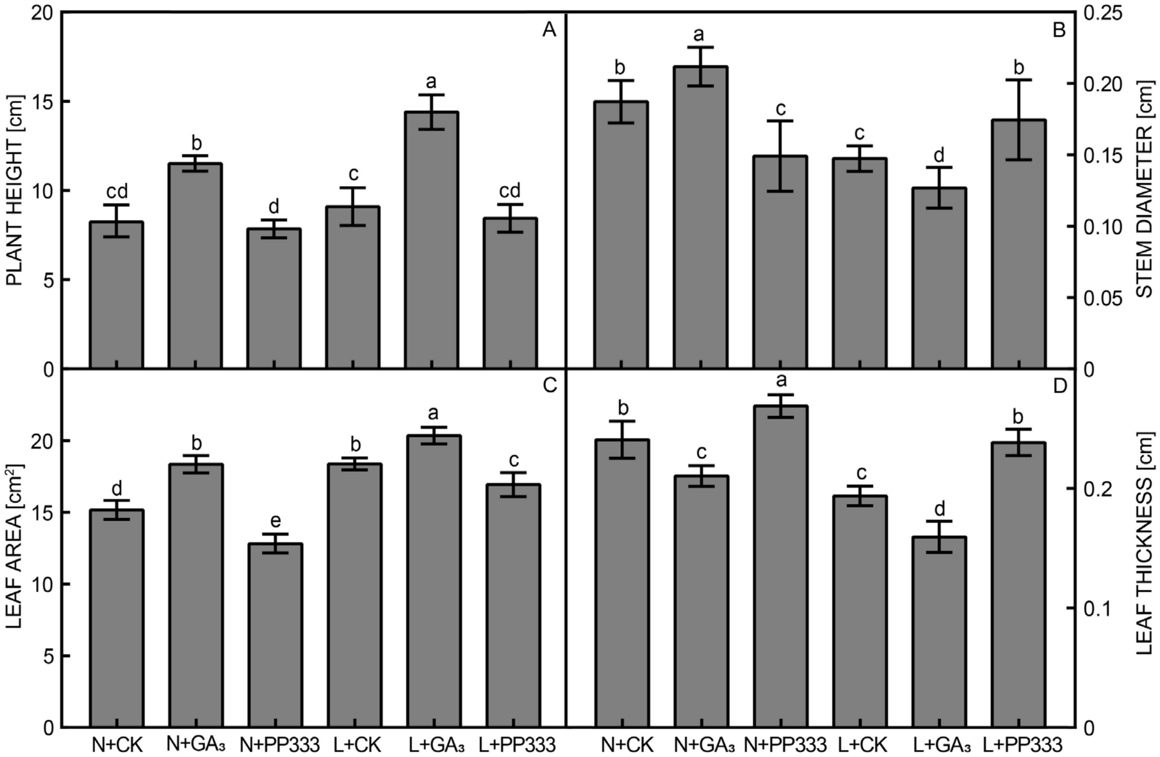

Fig. 1. Effects of low irradiance and GA_3 and PP333 treatments on growth indexes of pepper seedlings: *A* - plant height; *B* - stem diameter; *C* - leaf area; *D* - leaf thickness. Under normal irradiance, leaves were sprayed with distilled water (N+CK), under normal irradiance, leaves were sprayed with 100 mg dm^{-3} GA_3 (N+GA_3), under normal irradiance, leaves were sprayed with 50 mg dm^{-3} PP333 (N+PP333), under low irradiance, leaves were sprayed with distilled water (L+CK), under low irradiance, leaves were sprayed with 100 mg dm^{-3} GA_3 (L+GA_3), under low irradiance, leaves were sprayed with 50 mg dm^{-3} PP333 (L+PP333). Means ± SDs, $n = 6$, significant differences are marked by different lowercase letters (at $P < 0.05$) according to Duncan's multiple range test.

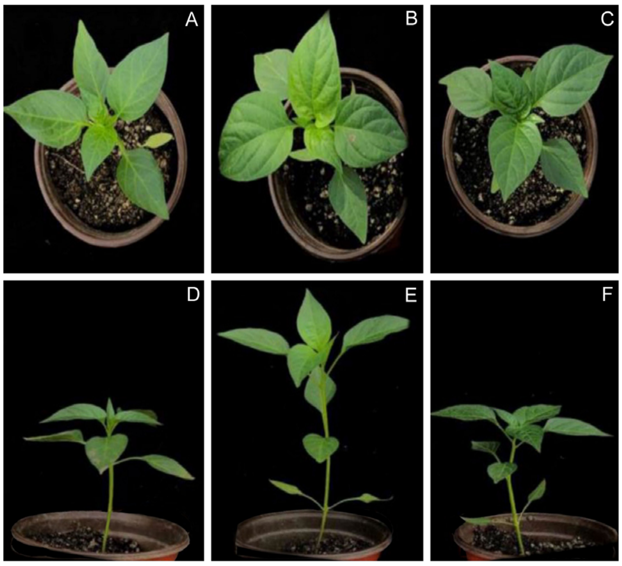

Fig. 2. The growth of pepper seedlings under different treatments: N+CK (*A,D*); L+CK (*B,E*); L+PP333 (*C,F*). Under normal irradiance, leaves were sprayed with distilled water (N+CK), under low irradiance, leaves were sprayed with distilled water (L+CK), under low irradiance, leaves were sprayed with 50 mg dm^{-3} PP333 (L+PP333).

irradiance on F_v/F_m and F_v/F_0 was similar. Low irradiance promoted the increase of F_v/F_m and F_v/F_0. The effect of GA_3 and PP333 on F_v/F_m and F_v/F_0 was not obvious under low irradiance, but GA_3 reduced F_v/F_m and F_v/F_0 under normal irradiance (Fig. 4).

ABS/CS_o, DI_o/CS_o, ET_o/CS_o, and TR_o/CS_o are specific activity parameters per unit cross-sectional area. Compared with normal irradiance, DI_o/CS_o and ET_o/CS_o decreased significantly under the shaded condition, while ABS/CS_o and TR_o/CS_o did not exhibit significant differences. Under normal irradiance, the spraying of GA_3 significantly increased the ratios of ABS/CS_o and DI_o/CS_o; the spraying of PP333 significantly increased the ratio of DI_o/CS_o but had no significant effect on other indexes. Under the shaded condition, the GA_3 significantly reduced the ET_o/CS_o ratio but had no significant effect on other indexes; there was no significant change in response to the spraying with PP333 (Fig. 5*A-D*).

ABS/RC, DI_o/RC, TR_o/RC, and ET_o/RC belong to the specific activity parameters in PS II reaction center. Compared with normal irradiance, ABS/RC and DI_o/RC were significantly decreased under the shaded condition, while ET_o/RC was significantly increased. The spraying of GA_3 significantly increased the ratios of ABS/RC, DI_o/RC, and TR_o/RC under normal irradiance and shaded conditions. GA_3 had no significant effect on ET_o/RC under normal irradiance but significantly increased ET_o/RC

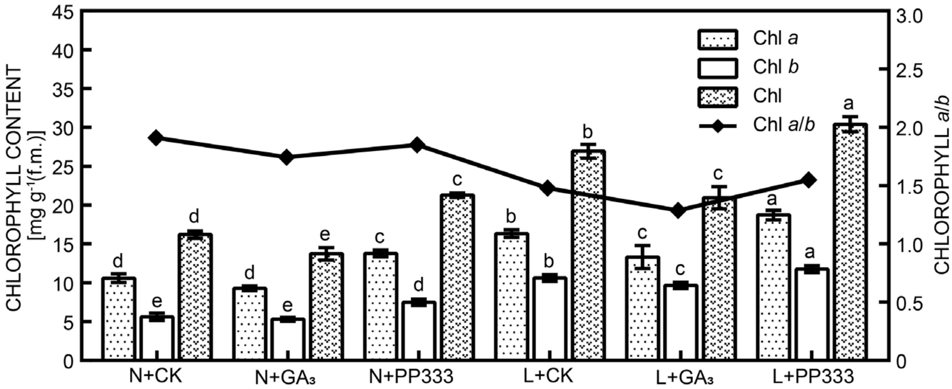

Fig. 3. Effects of low irradiance and GA_3 and PP333 treatments on the Chl *a*, Chl *b*, and Chl *a*+*b* content and Chl *a*/*b* ratio in pepper seedlings. Under normal irradiance leaves were sprayed with distilled water (N+CK), under normal irradiance leaves were sprayed with 100 mg dm^{-3} GA_3 (N+GA_3), under normal irradiance leaves were sprayed with 50 mg dm^{-3} PP333 (N+PP333), under low irradiance leaves were sprayed with distilled water (L+CK), under low irradiance leaves were sprayed with 100 mg dm^{-3} GA_3 (L+GA_3), under low irradiance leaves were sprayed with 50 mg dm^{-3} PP333 (L+PP333). Means ± SDs, $n = 6$, significant differences are marked by different lowercase letters (at $P < 0.05$) according to Duncan's multiple range test.

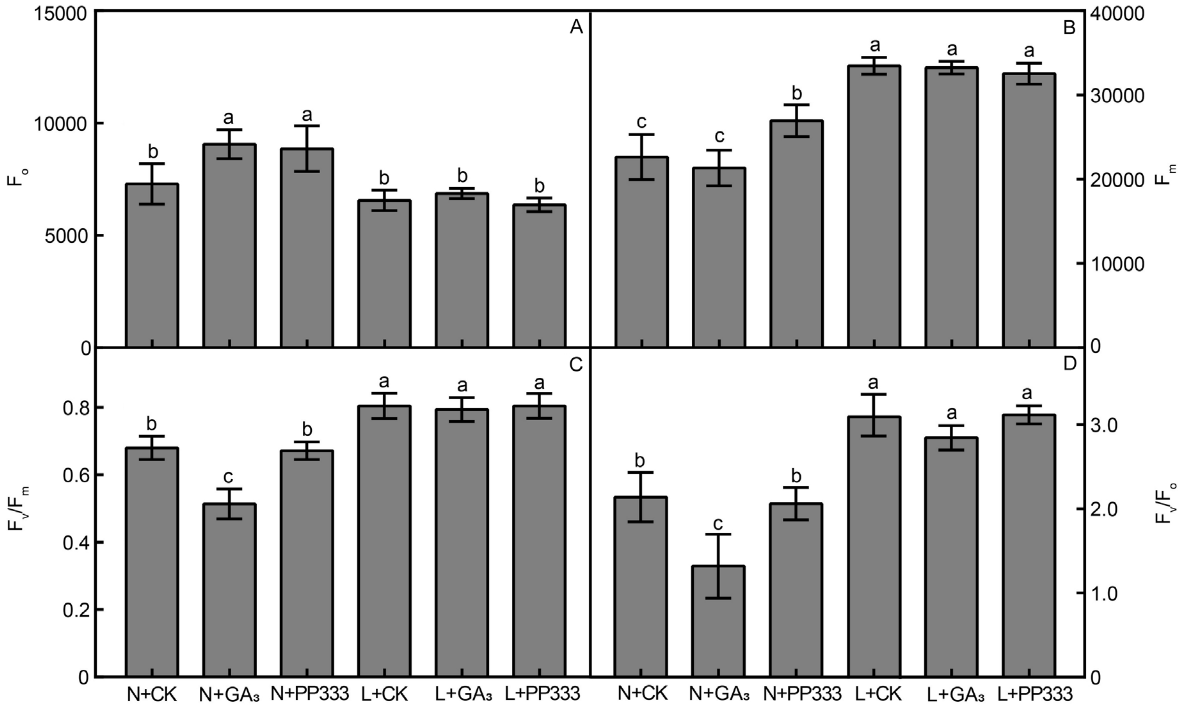

Fig. 4. Effects of low irradiance and GA_3 and PP333 treatments on the chlorophyll fluorescence parameters F_0, F_m, F_v/F_m, and F_v/F_0, which characterise photochemical efficiency of pepper seedlings. Under normal irradiance leaves were sprayed with distilled water (N+CK), under normal irradiance leaves were sprayed with 100 mg dm^{-3} GA_3 (N+GA_3), under normal irradiance leaves were sprayed with 50 mg dm^{-3} PP333 (N+PP333), under low irradiance leaves were sprayed with distilled water (L+CK), under low irradiance leaves were sprayed with 100 mg dm^{-3} GA_3 (L+GA_3), under low irradiance leaves were sprayed with 50 mg dm^{-3} PP333 (L+PP333). Means ± SDs, $n = 6$, significant differences are marked by different lowercase letters (at $P < 0.05$) according to Duncan's multiple range test.

under shaded conditions. There was no significant change in response to the spraying of PP333 (Fig. 5*E*-*H*).

The content of MDA in pepper seedlings, sprayed with distilled water under low irradiance, was the highest, while that in pepper seedlings, sprayed with GA_3 under normal irradiance, was the lowest. Under normal irradiance, the content of MDA in pepper seedlings sprayed with PP333 was significantly higher than the MDA content in seedlings sprayed with distilled water and GA_3. The content of MDA in pepper seedlings sprayed with distilled water under low irradiance was significantly higher than that of pepper seedlings sprayed with GA_3 and PP333. The content of MDA in pepper seedlings under low irradiance was significantly higher than that under normal irradiance (Fig. 6*A*).

The highest and lowest proline content were found in the pepper seedlings sprayed with GA_3 under low irradiance and in pepper seedlings sprayed with PP333 under normal

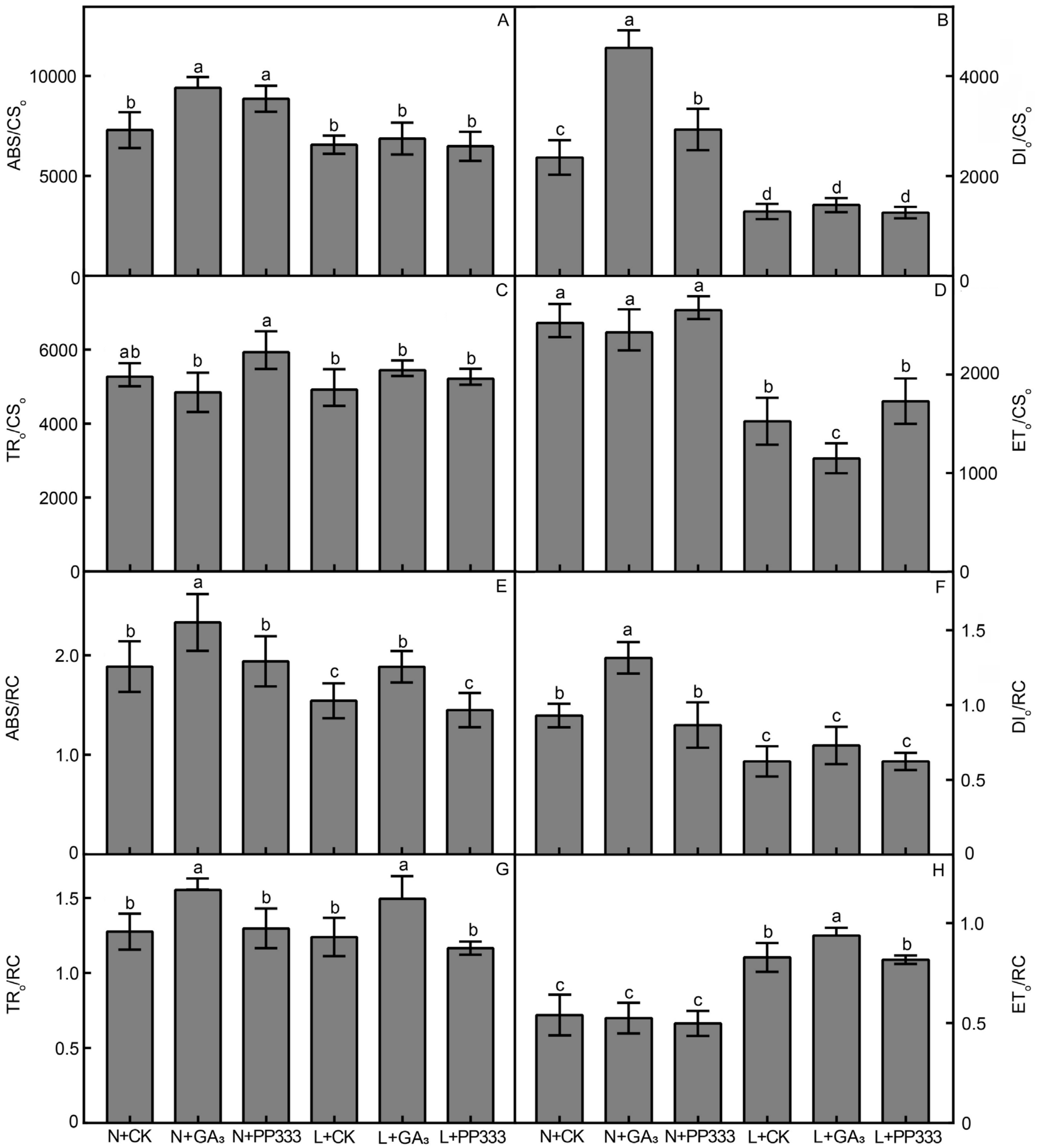

Fig. 5. Effects of low irradiance and GA_3 and PP333 treatments on specific activity parameters of pepper seedlings. Under normal irradiance leaves were sprayed with distilled water (N+CK), under normal irradiance leaves were sprayed with 100 mg dm^{-3} GA_3 (N+GA_3), under normal irradiance leaves were sprayed with 50 mg dm^{-3} PP333 (N+PP333), under low irradiance leaves were sprayed with distilled water (L+CK), under low irradiance leaves were sprayed with 100 mg dm^{-3} GA_3 (L+GA_3), under low irradiance leaves were sprayed with 50 mg dm^{-3} PP333 (L+PP333). Means ± SDs, $n = 6$, significant differences are marked by different lowercase letters (at $P < 0.05$) according to Duncan's multiple range test.

irradiance. Under normal irradiance, the proline content of the pepper seedlings sprayed with PP333 was significantly lower than that of pepper seedlings sprayed with distilled water and GA_3. The proline content of pepper seedlings under low irradiance was significantly higher than that under normal irradiance under the same treatment (Fig. 6*B*).

The soluble protein content of pepper seedlings sprayed with distilled water under normal irradiance was the highest, while that of pepper seedlings sprayed with distilled water under low irradiace was the lowest. Under normal irradiance, spraying GA_3 and PP333 significantly reduced the content of soluble protein in pepper seedlings. Under low irradiance, spraying GA_3

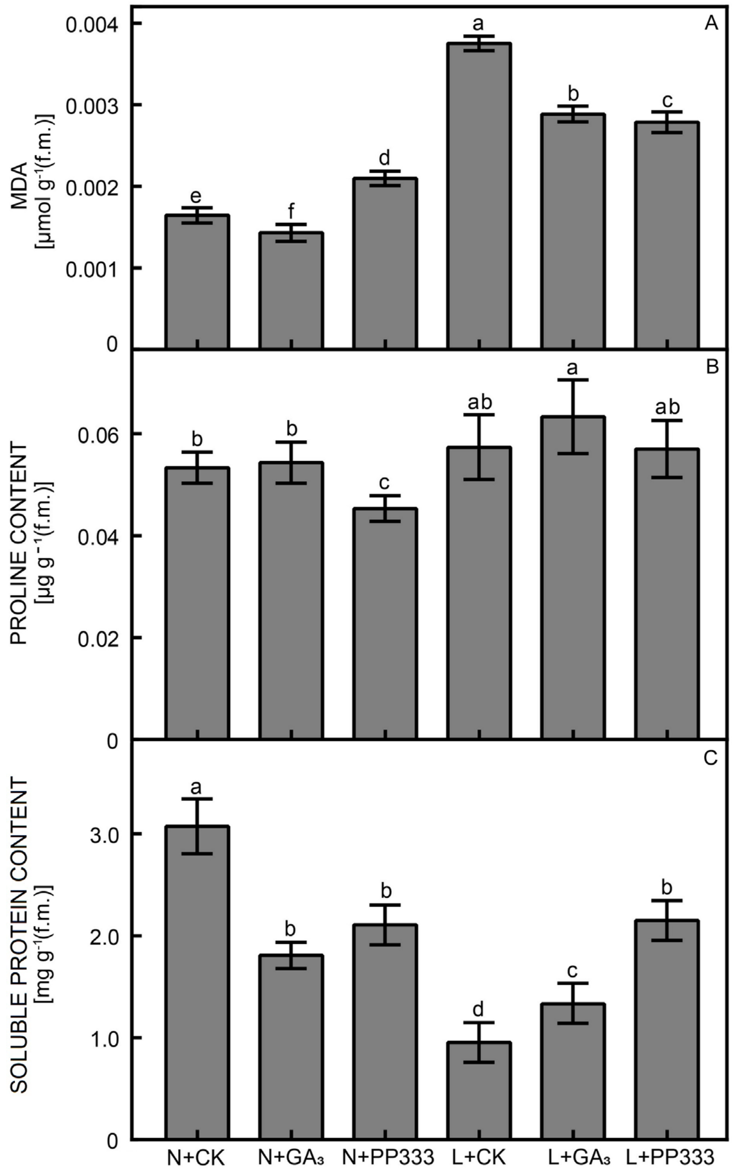

Fig. 6. Effects of low irradiance and GA_3 and PP333 treatments on content of malondialdehyde, proline, and soluble protein in pepper seedlings. Under normal irradiance leaves were sprayed with distilled water (N+CK), under normal irradiance leaves were sprayed with 100 mg dm^{-3} GA_3 (N+GA_3), under normal irradiance leaves were sprayed with 50 mg dm^{-3} PP333 (N+PP333), under low irradiance leaves were sprayed with distilled water (L+CK), under low irradiance leaves were sprayed with 100 mg dm^{-3} GA_3 (L+GA_3), under low irradiance leaves were sprayed with 50 mg dm^{-3} PP333 (L+PP333). Means ± SDs, $n = 6$, significant differences are marked by different lowercase letters (at $P < 0.05$) according to Duncan's multiple range test.

and PP333 significantly increased the content of soluble protein in pepper seedlings. The spraying of exogenous GA_3 and PP333 inhibits the soluble protein content of pepper seedlings under normal irradiance (Fig. 6*C*).

Discussion

Low irradiance (100 μmol m^{-2} s^{-1}) promoted an increase in plant height and leaf area, decrease in stem diameter and leaf thickness. Under normal or low irradiance, GA_3 increased plant height and the leaf area of the pepper seedlings but decreased leaf thickness. In contrast to GA_3, PP333 alleviated the effects of low irradiance on pepper seedlings. Under low irradiance (100 μmol m^{-2} s^{-1}), increase in the specific leaf area is a reaction mechanism to resist insufficient irradiance in plant. Plants capture more light by increasing the specific leaf area of their leaves, thus increasing the photosynthetic rate to make organic matter. Increase in the specific leaf area, which is a protective mechanism, can create organics in pepper by increasing the photosynthetic rate by capturing more irradiance, and decrease the respiration rate per leaf area (Kitao *et al.* 2000, Valladares *et al.* 2000). Consistent with the findings of Falcioni *et al.* (2017), the increase in leaf area induced by GA_3 supplementation, provided a higher irradiance-capturing surface for plants, resulting in thinner leaves, and increased leaf irradiance transmittance (Brodersen *et al.* 2010). The results of Falcioni's study also showed that plantlets subjected to PP333 had two layers of palisade-parenchyma. The enhanced light absorption by increasing the pigment content per leaf area could not compensate for the decreased light absorption due to the decrease of leaf area. However, the opposite phenomenon appeared in the plants supplemented with GA_3 (Falcioni *et al.* 2017).

The measurement of Chl fluorescence is proven to be a quantitative, non-invasive, rapid, and powerful method of assessing the properties of the photosynthetic apparatus and the extent to which plants are affected by different types of environmental stresses (Van Kooten *et al.* 1990, Calatayud *et al.* 2004). As one of the most important fluorescence parameters, F_v/F_m reflects the maximum photochemical efficiency of PS II active sites in the dark. Greater F_v/F_m value results in higher light utilization efficiency and stronger ability of plants to adapt to low-light conditions (Fu *et al.* 2012). Variation trends of the maximum quantum yield of PS II (F_v/F_m) depended mainly on irradiance conditions; F_v/F_m under low irradiance is higher than that under normal irradiance. These results indicated that low irradiance could increase the primary activity and primary light energy conversion efficiency of PS II reaction center in pepper leaves (Sui *et al.* 2012). In this experiment, the F_v/F_m of pepper seedlings under the shaded condition was higher than that under normal irradiance, indicating that pepper seedlings improved irradiance energy utilization by improving the activity of PS II. Studies have shown that stress severely impairs the normal function of photosynthetic apparatus in leaves through the functions of electron donor side, electron acceptor side, and electron transport chain of PS II (Wang *et al.* 2014). ABS/CS_o, DI_o/CS_o, TR_o/CS_o, and ET_o/CS_o in the PS II reaction centers decreased, which may have been caused by degradation or inactivation of reaction centers, and such changes in reaction centers can also be regarded as a self-protection mode of plants (Feng *et al.* 2009). In this study, the decrease in ABS/RC may be due to the inactivation or cracking of some reaction centers per unit area of leaves caused by low irradiance, which did not enhance the activity of the remaining active reaction centers but reduced the efficiency of the remaining active reaction centers, while the increase in ET_o/RC may be due to the activation of the corresponding defense mechanism of the reaction centers. To some extent, GA_3 can increase the extent of damage caused by low irradiance to PS II. However, the effect of PP333 on the change in the PS II reaction center induced by low irradiance was not significant.

Studies have shown that bright irradiance (1 200 μmol m^{-2} s^{-1}) can destroy chloroplasts and cells, thus the Chl content was reduced in plant leaves (Sun *et al.* 2014). In contrast, under low irradiance, the number of chloroplasts per leaf area decreased, but the increase of individual chloroplasts led to an increase in leaf Chl content (Fu *et al.* 2012). The increase of Chl *b* under the low irradiance attributes to improved ability to absorb the radiation, and this physiological reaction makes pepper increase the light energy absorption of leaves under low irradiance (Chen *et al.* 1998). The results showed that low irradiance and exogenous PP333 could increase the content of Chl, while exogenous GA_3 could decrease the content of Chl; low irradiance and exogenous GA_3 reduced the Chl *a*/*b* ratio, while exogenous PP333 increased the Chl *a*/*b* ratio (Fig. 3). The decrease in the Chl content caused by exogenous GA_3 may be due to the fact that increases in leaf area caused by GA_3 may lead to Chl dilution (Mbandlwa *et al.* 2020).

Peroxidation of lipids caused by excess of reactive oxygen species increases the permeability of cell membranes and destruction of their functions (Zhang *et al.* 2012). MDA is the final decomposition product of membrane lipid peroxidation, and its content can reflect the degree of plant stress injury. MDA has been widely used to detect the damage degree of oxide film. To a certain extent, PP333 restored the adverse reactions caused by stress, such as reducing MDA content and increasing the content of proline and soluble protein (Hajihashemi *et al.* 2013).

The accumulation of soluble substances, which contribute to osmotic regulation, is also closely related to the pressure potential of cells. The increase in soluble substances can not only reduce the water potential of cells but can also prevent the inactivation of biological macromolecules, so as to reduce the damage caused by low temperature. Accumulation of osmoregulatory substances is one of the important ways for plants to resist cold injury. A large number of studies have found that osmoregulatory substances related to cold resistance of plants mainly include soluble sugars, free amino acids, and soluble proteins (Jiang *et al.* 2015).

Conclusion

Pepper seedlings under low irradiance will produce abnormal growth phenomenon of longer stalks and thinner leaves, easy to lodging. This study found that PP333 increased stem diameter and leaf thickness, increased the content of anti-stress substances in pepper seedlings, promoted the growth of pepper seedlings under low irradiance, and improved their resistance to low irradiance. These results provide a basis for improving pepper production facilities.

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