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Full Length Research Paper

Effects of different red to far-red radiation ratios on the senescence of greenhouse chrysanthemum leaves

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Red (660±30 nm) and far-red (730±30 nm) LED light sources were combined in different proportions to yield a design with four R:FR ratio treatments (0.5, 2.5, 4.5 and 6.5). Chrysanthemum plants irradiated with natural light served as the control (R:FR=1.02). The chlorophyll a (Chla) content and SPAD value of chrysanthemum leaves were highest for an R:FR ratio of 2.5 and lowest for an R:FR ratio of 6.5. The chlorophyll b (Chlb) and carotenoid content were highest for an R:FR ratio of 0.5 and lowest for R:FR ratios of 6.5 and 2.5, respectively. The superoxide dismutase (SOD), peroxidase (POD) activity and soluble protein content of the chrysanthemum leaves were highest for an R:FR ratio of 2.5, whereas the catalase (CAT) activity was highest for an R:FR ratio of 4.5. The SOD, POD, CAT activity and soluble protein content were found to be lowest for an R:FR ratio of 0.5. The malondialdehyde (MDA) content of the chrysanthemum leaves was highest for an R:FR ratio of 0.5 and lowest for an R:FR ratio of 2.5. These results implied that the antioxidant enzyme activity and the anti-senescence ability of the chrysanthemum leaves were highest for an R:FR ratio of 2.5 and lowest for an R:FR ratio of 0.5.

Key words: Chrysanthemum, R:FR, senescence, chlorophyll, antioxidant enzyme.

INTRODUCTION

Regulation of the growth, development and quality of crops by the control of light quality has attracted extensive attention worldwide (Segovia and Figueroa, 2003; Li and Chinnappa, 2004; Raik et al., 2008). Previous studies have confirmed that phytochrome, which is most sensitive to red light and far-red light, is involved in light signal induction, antioxidant metabolism and the regulation of the senescence process (Biswal and Basanti, 1984; Guiamet, 1989; Polidoros and Scandalios, 1997). It was reported that increasing the proportion of red light accelerated plant cell division and tissue growth (Latkowska et al., 2000), promoted the growth of the plant stem and the root system (Onofrio et al., 1998; Simonovic et al., 2000), and improved the photosynthetic rate (Yanagi and Okamoto, 1997; Pu et al., 2005), the dry matter accumulation and the dry matter distribution to sink organs (Yorio et al., 2001; Leonid et al., 2007; Danguole et al., 2011).

The reduction of chlorophyll and soluble protein content is hypothesised to be a typical characteristic of leaf senescence (Gan and Amasino, 1997; Corpas et al., 2001). Heraut et al. (1999) showed that red light could increase the chlorophyll content in leaves. Li and Kubot (2009) found that the anthocyanins, carotenoid and chlorophyll content in lettuce decreased with an increase of the far-red component in the light spectrum. The senescence of plant leaves can be attributed to the decrease in protective enzyme activities and the increase of reactive oxygen species (ROS) (Pastori and del Rı´o, 1997; Procha´zkova et al., 2001). Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are important protective enzymes that can scavenge ROS in plants, prevent the peroxidation of membrane lipid and delay the senescence process (Kaiser, 1976; Pastori and del Rı´o, 1997). Malondialdehyde (MDA) is the product of

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membrane lipid peroxidation, which reflects the degree of cell injury caused by ROS (Dhindsa et al., 1982). Pu et al. (2005) reported that the POD, SOD and APX (ascorbate peroxidase) activities of tomato leaves treated with red light and blue light were higher than those treated with white light. Wang et al. (2010) showed that purple light and blue light induced the expression of the antioxidant enzyme gene as well as increase antioxidant enzyme activities, slowed down the decrease of chlorophyll and soluble protein, and maintained MDA at a low level. Green light, yellow light and red light inhibited antioxidant enzyme activities and caused a decrease of chlorophyll and soluble protein content, whereas these light treatments increased the MDA content and accelerated the senescence process of cucumber plants.

Chrysanthemum is one of the four most important cut flowers in the world as well as China's main export flower. However, no reports have addressed the effects of different R:FR ratios on the senescence characteristics of chrysanthemum leaves. Because it has been inferred from previous research that the threshold R:FR ratio for promoting or delaying flower bud differentiation was 5.3 and the critical R:FR ratio for promotion or delay of visible buds was approximately 0.5 (Yamada et al., 2009), we designed an experiment with four R:FR ratio treatments, that is, 0.5, 2.5, 4.5 and 6.5, to study the dynamic effects of different R:FR ratios on the senescence characteristics of chrysanthemum leaves. The results were expected to provide a scientific basis for the use of light quality to regulate the growth and development of chrysanthemums.

MATERIALS AND METHODS

Experimental design

The experiment was conducted in the experimental greenhouse of Nanjing University of Information Science and Technology from October 2010 to February 2011. The greenhouse was 9.6 m wide and 30.0 m long. Its top height and shoulder height were 5.0 m and 4.5 m, respectively. The experimental material was chrysanthemum (*morifolium Ramat, cv.* 'Jingba'). The plants were transplanted on 6 October, when the seedlings were approximately 20 cm high with 6 to 10 leaves. A mixture of vermiculite and perlite with a volume ratio of 2:1 was used as the culture substrate. The planting space was 20 cm × 20 cm in size. During the vegetative growth phase, fluorescent lamps (photosynthetically active radiation (PAR) = 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) were used to supplement the light for 5 h (18:00 to 23:00) to extend the illumination time. When the plant height reached 50 cm, natural light was shaded using black plastic film, and LED light sources with different R:FR ratios were used to produce short-day treatments. Each LED light source consisted of 360 evenly and proportionally arranged red and far-red LED lamps. The red (R: 660±30 nm): far-red (FR: 730±30 nm) energy ratios of the four experimental treatments were 0.5, 2.5, 4.5 and 6.5. The LED light sources (50 cm long, 50 cm wide) were fixed 15 cm above the canopy. A 10 h day length (08:00 to 17:00) was used for the short-day treatments. Plants irradiated with natural light (R:FR=1.02) served as the control (CK). The photosynthetically active radiation (PAR) of all treatments was regulated to be 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the top of the canopy. Each treatment had 30 chrysanthemum plants. All plants were irrigated with a nutrient solution with a conductivity of 1.5 $ms \cdot cm^{-1}$ (200 $\mu g \cdot g^{-1}$ N; 80 $\mu g \cdot g^{-1}$ P; 170 $\mu g \cdot g^{-1}$ K).

Determination of photosynthetic pigment content

The 5th to 8th functional leaves from the top of the plant were picked between 9:00 to 11:00 am on days 15, 30, 45 and 60 of the treatments and placed in 96% ethanol for 48 h until the chlorophyll was completely extracted. The chlorophyll a (Chla), chlorophyll b (Chlb) and carotenoid contents were determined with the method used by Hugo et al. (2008). The SPAD value of the leaves was determined with a chlorophyll meter (SPAD502, Knioca Minolta, Japan).

Determination of enzyme activities, MDA and soluble protein content

For the determination of enzyme activities, MDA and soluble protein content, the leaves picked for analysis were quickly frozen with liquid nitrogen and stored in a -40°C ultra-low-temperature freezer. All samples were analysed in the laboratory after the sampling was completed. SOD activity was determined according to the method proposed by Rabinowitch and Sklan (1980) and the amount of enzyme inhibiting 50% of the nitroblue tetrazolium (NBT) reduction per hour was taken as one enzyme activity unit. CAT activity was determined with the UV absorption method (Chance and Maehly, 1955) and expressed as the decrease of OD_{240} per minute. POD activity was determined with the guaiacol method (Li, 2000) and expressed as the increase of OD_{470} per minute. The MDA content was determined with the method proposed by Zhao et al. (1994). The soluble protein content was determined with the coomassie brilliant blue-G250 staining method (Bradford, 1976).

Data analysis

The data were analysed in Microsoft Office Excel 2003. The activities of SOD, POD, and CAT and the contents of photosynthetic pigment, MDA and soluble protein for all R:FR ratio treatments were statistically analysed using SPSS 15.0 (SPSS Science Inc., USA). The results of variance analysis were expressed by the error bars in each figure. These bars represented the standard deviations for three replications. A least significant difference test was used to analyse the significance of the differences between treatment means. The differences for which $p<0.05$ were considered significant.

RESULTS

Effects of different R:FR ratios on photosynthetic pigment content

Figure 1 shows the effects of different R:FR ratios on the Chla, Chlb, and carotenoid content and SPAD value of the chrysanthemum leaves. Generally, the Chla, Chlb, and carotenoid content and SPAD value increased over time during the treatments. The Chla content and SPAD value of the chrysanthemum leaves for an R:FR ratio of 2.5 were the highest among all the treatments (Figure 1A and 1D). However, the carotenoid content for this treatment was the lowest (Figure 1C). The Chlb content was highest for an R:FR ratio of 0.5 and lowest for an R:FR ratio of 6.5 (Figure 1B).

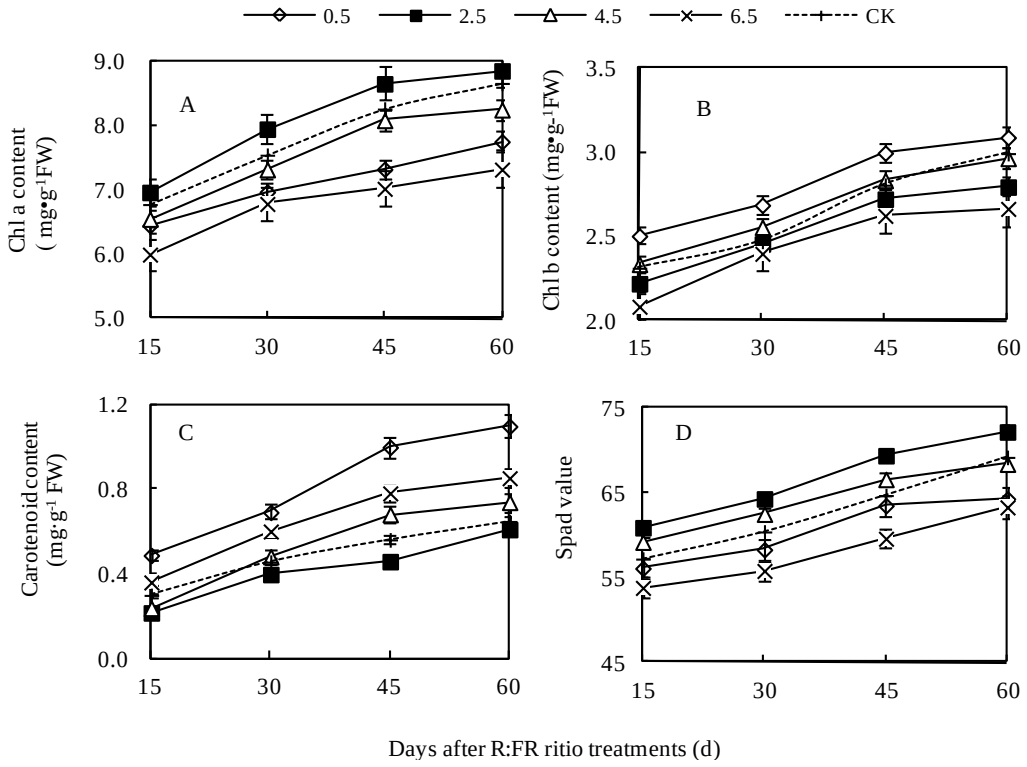

Figure 1. Effects of different R:FR ratios on photosynthetic pigment content of chrysanthemum leaves.

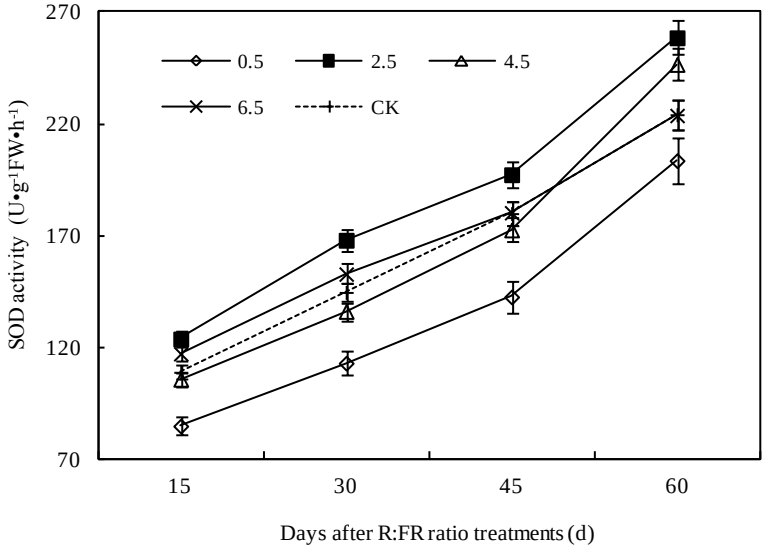

Figure 2. Effects of different R:FR ratios on SOD activity of chrysanthemum leaves.

Effects of different R:FR ratios on SOD enzyme activity

Figure 2 shows that SOD activity tends to increase over time and all treatments produce consistent trends. The SOD activity of the chrysanthemum leaves was highest for an R:FR ratio of 2.5 and lowest for an R:FR ratio of 0.5. On the 45th day of exposure, the treatments with

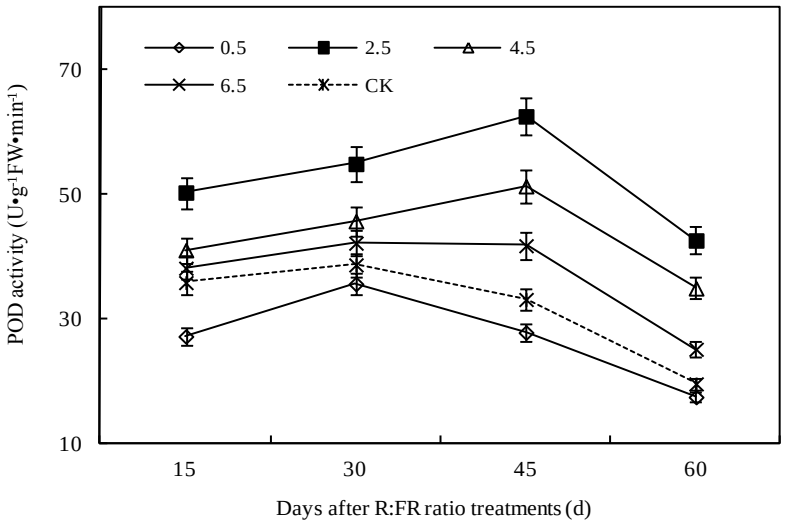

Figure 3. Effects of different R:FR ratios on POD activity of chrysanthemum leaves.

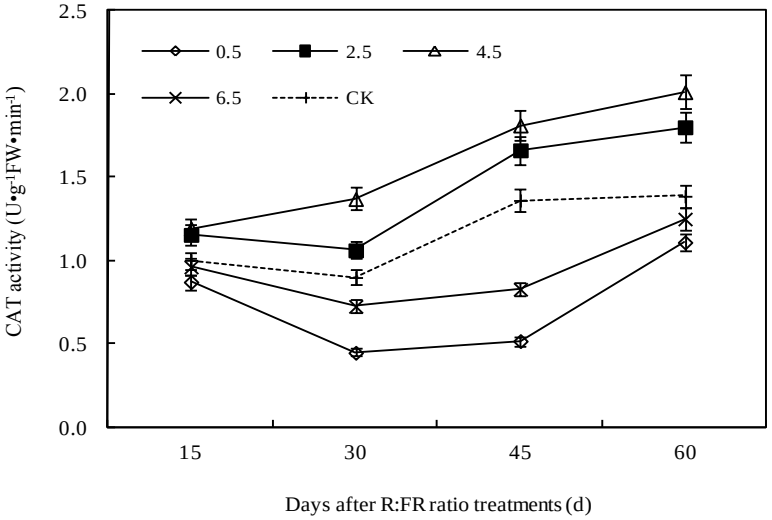

Figure 4. Effects of different R:FR ratios on CAT activity of chrysanthemum leaves.

R:FR ratios of 6.5 and 4.5 did not differ significantly in SOD activity from CK. On the 60th day of the treatments, the SOD activity of the leaves treated with an R:FR ratio of 4.5 was markedly higher than the activity found for an R:FR ratio of 6.5 and CK, while no significant difference in SOD activity was found between the leaves treated with an R:FR ratio of 6.5 and CK.

Effects of different R:FR ratios on POD enzyme activity

Figure 3 shows that the POD activity of chrysanthemum leaves under various treatments first increases and then decreases over time. The POD activity of the chrysanthemum leaves in the five R:FR ratio treatments showed a descending order corresponding to 2.5>4.5>6.5> CK>0.5. Significant differences were found among the different treatments.

Effects of different R:FR ratios on CAT enzyme activity

Figure 4 shows the CAT activity of chrysanthemum leaves exposed to different R:FR ratios. All treatments followed the same trend over time. The CAT activity of the chrysanthemum leaves for R:FR ratios of 4.5 and 2.5

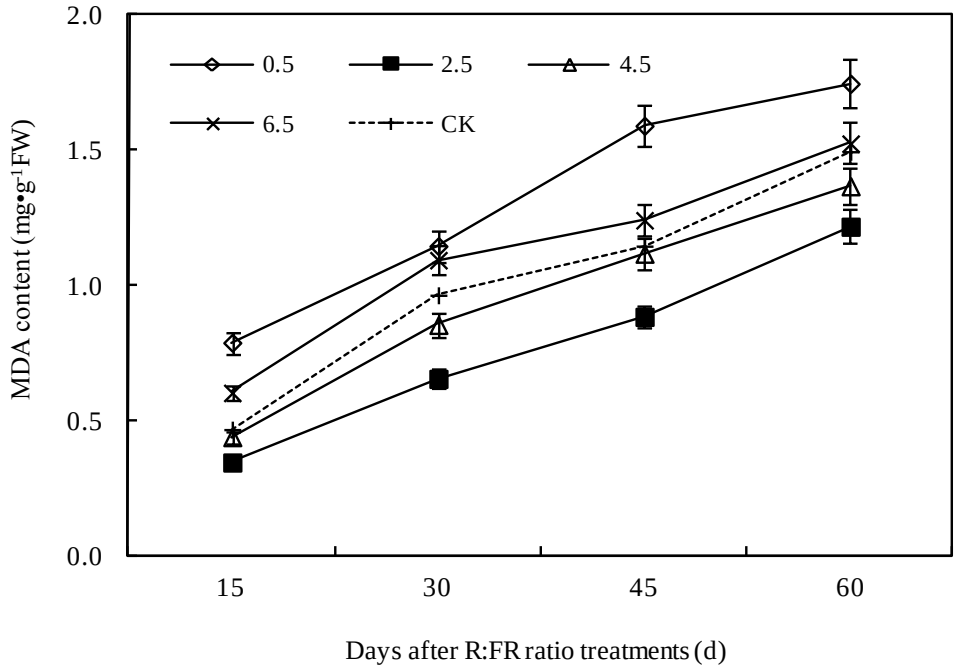

Figure 5. Effects of different R:FR ratios on MDA content of chrysanthemum leaves.

was significantly higher than that for CK, with the ratio of 4.5 always at the highest level. However, the CAT activity of chrysanthemum leaves for R:FR ratios of 6.5 and 0.5 was significantly lower than that for CK, with the ratio of 0.5 always at the lowest level. The average CAT activity of chrysanthemum leaves for R:FR ratios of 4.5 and 2.5 was 37.49% and 22.26% higher, respectively, than that for the CK treatment. However, the average CAT activity for R:FR ratios of 6.5 and 0.5 was 19.08% and 36.78% lower, respectively, than that for CK.

Effects of different R:FR ratios on MDA content

The effects of different R:FR ratios on the MDA content of the chrysanthemum leaves are shown in Figure 5. The MDA content of the leaves increased over time for all treatments. The sequence of R:FR ratios corresponding to decreasing values of the MDA content under different treatments was 0.5>6.5>CK>4.5>2.5. This result indicated that the level of injury of the cell membrane for an R:FR ratio of 0.5 was larger than that for the other treatments. The MDA content of the chrysanthemum leaves for R:FR ratios of 4.5 and 6.5 did not differ significantly from that for CK, whereas that for an R:FR ratio of 2.5 was markedly lower than those for the other treatments. This results indicated that the degree of membrane lipid peroxidation as well as the degree of senescence of the leaves were slight in the treatment with an R:FR ratio of 2.5.

Effects of different R:FR ratios on soluble protein content

The effects of different R:FR ratios on the soluble protein content are shown in Figure 6. The soluble protein content tended to increase continuously over time. During the entire time of exposure, the soluble protein content for an R:FR ratio of 2.5 was markedly higher than that for CK. There was no significant difference in soluble protein content between the leaves exposed to an R:FR ratio of 4.5 and CK after 45 days of treatment. The soluble protein content of the leaves for R:FR ratios of 6.5 and 0.5 was clearly lower than that for CK. The minimum soluble protein content was found in the treatment with an R:FR ratio of 0.5.

DISCUSSION

The senescence of plant leaves can be characterised by the degradation of chlorophyll and proteins, the conversion of peroxisomes into glyoxysomes, and an increase in the production of reactive oxygen species (Gan and Amasino, 1997; Corpas et al., 2001). SOD, POD and CAT activities reflect the physiological activity of plants (Procha´zkova et al., 2001), and MDA content reflects the degree of membrane lipid peroxidation. Rubisco is a main component of soluble protein. It has been shown that the degradation of rubisco indicates the senescence of leaves (Martin and Thimann, 1972;

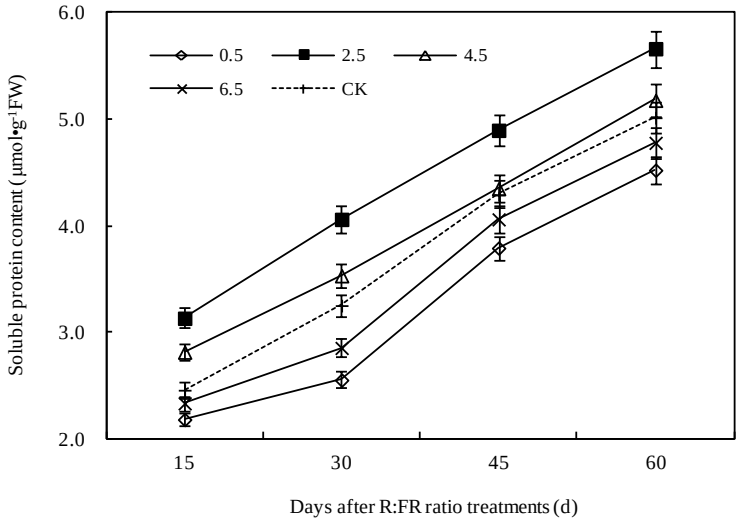

Figure 6. Effects of different R:FR ratios on soluble protein content of chrysanthemum leaves.

Wittenbach, 1979). The present study showed that during the treatment period, the Chla content, the SPAD value, the activities of SOD, POD and CAT, and the soluble protein content in the chrysanthemum leaves for an R:FR ratio of 2.5 were clearly higher than those for CK. However, the Chlb, carotenoid and MDA contents for this treatment were significantly lower than those for CK. Evidently, the chrysanthemum leaves treated with an R:FR ratio of 2.5 were more capable of scavenging active oxygen and free radicals. This capability allowed them to reduce the level of injury to their cell membrane system and thus delayed the process of senescence. For chrysanthemum leaves treated with an R:FR ratio of 0.5, the Chla content, the SPAD value, the SOD, POD and CAT enzyme activities, and the soluble protein content were significantly lower than those for CK, whereas the Chlb, carotenoid and MDA contents were markedly higher than those for CK. This result suggested that an R:FR ratio of 0.5 accelerated the senescence of the chrysanthemum leaves. The results of this study were consistent with the findings of previous research that increasing the proportion of far-red light increased the degree of senescence of the leaves, whereas increasing the proportion of red light inhibited leaf senescence (Biswal and Basanti, 1984; Pu et al., 2005; Wu et al., 2007).

Phytochrome is involved in light signal induction, antioxidant metabolism and the regulation of the senescence process (Biswal and Basanti, 1984; Guiamet et al., 1989; Polidoros and Scandalios, 1997) and is most sensitive to red light and far-red light. In this experiment, the protective enzyme activities of chrysanthemum leaves treated with different R:FR ratios were significantly different from those of CK. This difference may be related to the phytochrome regulation of antioxidant enzyme gene expression and needs further study. In our study, it was observed that an increase in the proportion of red light to produce R:FR ratios higher than 2.5 decreased the SOD, POD and CAT activities of the chrysanthemum leaves and promoted the senescence of the leaves. These effects may be related to the induction of inhibition cofactor activity for indoleacetic acid oxidase (IAAO) by red light (Mumford et al., 1961; Poudel et al., 2008).

Conclusions

In the four mentioned R:FR ratio treatments, the antioxidant enzyme activity and the anti-senescence ability of the chrysanthemum leaves were highest for an R:FR ratio of 2.5 and lowest for an R:FR ratio of 0.5. An increase in the R:FR ratio to 4.5 or 6.5 decreased the anti-senescence ability of chrysanthemum leaves compared with an R:FR ratio of 2.5. The results of this study were expected to provide a scientific basis for selecting different R:FR ratios to regulate the growth and development of greenhouse chrysanthemums.

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