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SHORT COMMUNICATION

Comparison of jasmine antioxidant system responses to different degrees and durations of shade

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Abstract

Jasmine [*Jasminum sambac* (L.) Aiton] growth and development is affected by long-term shade. To determine the effects of short-term shade on jasmine physiology, the contents of soluble proteins, malondialdehyde (MDA) and antioxidative enzymes were comparatively investigated during 24 h (short-term, ST) and 7 days (medium-term, MT) of varying light regimes. The results showed that the protein content exhibited two peaks under ST treatment, and shade postponed the first peak 2 h later than full light. On the whole, protein synthesis was reduced by ST shade and induced by MT shade, whereas MDA content decreased during all shade treatments. Under ST shade, superoxide dismutase (SOD), peroxidase (POD) and ascorbate peroxidase (APX) activities were enhanced, but catalase (CAT) activity was repressed by limited irradiances. However, the antioxidant enzymes responded differently—SOD activity increased after 2 h of shading and then kept on a high level, whereas POD, APX and CAT activities increased mainly during the first hour and deceased subsequently. Under MT shade, these antioxidative enzymes responded differently to varying light irradiances, too. In general, POD and CAT activities were repressed, SOD activity was induced by weak (50% irradiance) shade and reduced by moderate (20% irradiance) and severe (5% irradiance) shade. APX activity was rather more complicated and irregularly responded to different degrees and durations of shade, meaning it might not be the main enzyme to remove ROS in jasmine plants under shading condition. The increase protein content with prolonged shade represents the sound adaptive ability of jasmine plants to restricted irradiances. At the same time, rapid changes in proteins and antioxidants reflect the efficient metabolic apparatus of the plant in response to shade. Therefore, the jasmine cultivar is shade tolerant. Furthermore, shade could help the plants protect themselves from full light, and some degrees of shade were beneficial to their antioxidant system. However, severe shade (5% of irradiance) is only suggested for a few hours to protect the plants at solar noon. If the plants are continuously shaded for 3–7 days, weak (20%) to moderate (50%) level of irradiance should be applied.

Keywords *Jasminum sambac* · Antioxidative enzyme · Ascorbate peroxidase · Catalase · Peroxidase · Shade tolerance · Superoxide dismutase

Abbreviations

APX	Ascorbate peroxidase
CAT	Catalase
DOS	d of shading
HOS	h of shading
MDA	Malondialdehyde
MT	Medium-term
POD	Peroxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase
ST	Short-term

Introduction

Jasmine (*Jasminum sambac* [L.] Aiton) is an important horticultural crop throughout the world. Its absolute and essential oils are broadly used in the perfume industry for their sweet and elegant fragrance (Edris et al. 2008). Jasmine has been cultivated for nearly 2000 years in China because of its high value for scenting a specific 'jasmine tea' and its use in traditional Chinese medicine (Deng et al. 2016, 2017). Recently, jasmine plants have been popularly potted

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in the greenhouse as ornamental flowers. Thus, providing suitable environments for these plants is necessary to optimize their growth. This is particularly important in eastern China, where the flowering of the species peaks during the hot summer months (Deng et al. 2012a, b). Under these conditions, the plants must be shaded by commercial plastic shading nets to avoid damage from high temperature and strong irradiance.

Shade has been demonstrated to be a direct impact factor on jasmine growth and development, affecting the morphology, anatomy, flowering, and photosynthesis-related characters, including photosynthetic capacity, chlorophyll fluorescence parameters and photosynthetic pigment contents (Arunachalam and Reddy 2007; Deng et al. 2012a, b). Studies have shown that unshaded (full sunlight) and severely shaded (5% of natural irradiance) plants suffer from photoinhibition and light deficiency, respectively, and that weak (20% of natural irradiance) to moderate (50% of natural irradiance) degrees of shading were beneficial to plant growth, development and blooming (Deng et al. 2012a, b). Therefore, insight into the physiological and biochemical reactions of jasmine plants to shading is meaningful for better production management.

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen and include hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\cdot -}$), hydroxyl radical ($OH^{\cdot}$), hydroperoxyl radical ($HO_2^{\cdot}$), alkoxy radical ($RO^{\cdot}$) and singlet oxygen ($^1O_2$) (Yordanova et al. 2003; Karuppanapandian et al. 2011). ROS develop as a natural by-product of the normal oxygen metabolism in plants and have important actions in cell homeostasis and signaling pathways (Sharma and Dubey 2007; Karuppanapandian et al. 2011). However, high levels of ROS dramatically induced during environmental stress (e.g., excessive irradiance) can result in significant damage to cell structures and the degradation of lipids, proteins and nucleic acids, further leading to leaf chlorosis and senescence (Rout and Shaw 2001; Yordanova et al. 2003; Maffei et al. 2007). Under normal conditions, cells defend themselves against ROS damage by maintaining ROS at a low level through the action of various antioxidant enzymes such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), peroxidases (POD; EC 1.11.1.7) and ascorbate peroxidase (APX; EC 1.11.1.11) (Foyer and Noctor 2005; Maffei et al. 2007). Thus, high levels of SOD, POD, APX and CAT are critical for the survival of many plant species under environmental stresses, including shade (Peltzer and Polle 2001). Jasmine plants have shown that both POD and SOD activities are induced, CAT activity is repressed, and APX activity is irregular during 35 days of long-term shade (Deng et al. 2012b). Changes in the activities of these antioxidative enzymes are among the first observable physiological responses to shade (Peltzer and Polle 2001; Favaretto et al. 2011). Furthermore, as an important indicator of cellular membrane lipid peroxidation responded to abiotic stresses, the content of malondialdehyde (MDA) can be used to evaluate the oxidative degree and explain the results on antioxidant enzymes to some extent (Sharma and Dubey, 2007; Deng et al. 2012a). Therefore, revealing the underlying characteristics of these enzymes and MDA during short periods of limited irradiance is helpful for a better understanding of jasmine shade tolerance.

This study was designed to determine the effects of varying light regimes on the antioxidant system of jasmine plants during short- and medium-term shade (24 h and 7 days, respectively) as reflected in the changes of protein, MDA and the antioxidative enzymes SOD, POD, APX and CAT. This information can contribute to the further understanding of how the species responds to shade, thereby providing a reference for improving its daily management.

Materials and methods

The most popular jasmine cultivar, 'Shuangbanmoli', was used in this experiment. Similar 3-year-old plants were subjected to four irradiance levels, 100% (non-shaded, control), 50% (weak shade), 20% (moderate shade) and 5% (severe shade) of natural irradiance, in net-houses (2.5 m in height, 3 m in length and 2 m in width) covered with one or two layers of commercial plastic shading nets. Each treatment involved 20 pots (0.15 m in height and 0.17 m in diameter), and every pot contained five plants ~ 0.25 m in height. The growth conditions and daily management were the same as those described by Deng et al. (2012a, b). The experiment was replicated three times.

Approximately 0.2 g of leaf samples were collected after 0, 1, 2, 4, 8, 12 and 24 h of shading (HOS) to detect the influence under ST light stress, beginning at 08:00. Another 0.2 g of leaf samples was collected after 0, 1, 2, 3, 4, 5, 6 and 7 days of shading (DOS) to detect the influence under MT light stress. The samples were frozen immediately at − 80 °C. Protein content was determined based on the method of Bradford (1976), employing bovine serum albumin as a standard. The activities of POD, SOD, APX and CAT were analyzed following the methods described by Deng et al. (2012b). One unit (U) of POD was expressed as 0.01 μmol of tetraguaiacol mg^{-1} protein min^{-1}, one U of SOD was expressed as ΔA_{560nm} per milligram of protein per minute, one U of APX was defined as the amount of enzyme required to degrade 1 μmol ascorbic acid mg^{-1} protein min^{-1}, and one U of CAT was defined as the amount of enzyme required to degrade 0.1 μmol H_2O_2 mg^{-1} protein min^{-1}. The content of MDA was determined following the method of thiobarbituric acid, as described by Deng et al. (2011).

Statistical analysis was conducted with one-way analysis of variance (ANOVA) using the statistical software package SPSS 17 for Windows, and Duncan's multiple range test was employed to detect differences between the means of the data (with P set at 0.05 or 0.01).

Results and discussion

Protein content

Under ST shading, soluble protein contents changed slightly in all plants during the first hour. However, the values increased rapidly at the beginning of 1 HOS and reached the first peak after 2 (in unshaded plants) or 4 (in shaded plants) HOS (Fig. 1a). Then, all of the values decreased until 8 HOS but increased again by 12 HOS (Fig. 1a). At 24 HOS, the values were nearly the same as those at the beginning of the treatment (Fig. 1a). The results showed that the protein contents exhibited two peaks within 24 HOS, but the shading treatment postponed the first peak 2 h later than the unshaded control did. The two peaks may be closely related with the midday depression of photosynthesis in plants under strong irradiance and high temperature, which reduced protein synthesis (Guo et al. 2015). In the present study, shade not only postponed the depression, but also shortened the depression time about 2 h (Fig. 1a). Undoubtedly, this is beneficial to jasmine plant growth. However, at other time points (particularly at 2 and 12 HOS), the protein contents in unshaded plants were higher than shaded ones. This may be resulted by the natural weak sunshine irradiance during the early morning and evening. Therefore, daily shading should be applied for only a few hours. To the severe level (5% irradiance) of shade, it is reasonable from 10:00 to 16:00.

Under MT shading, the protein contents changed slightly during the first 4 days but clearly increased thereafter till the end (Fig. 1b). Although all plants showed similar changing tendencies, different degrees of shading exhibited different effects. The values of 5% irradiance were always significantly higher than those of other treatments at 4–7 DOS (Fig. 1b). On the whole, the protein contents increased with both the degree and time of shading. Therefore, the jasmine cultivar exhibited good shade tolerance during the period in the present study, which was similar to the performance of another double-petal jasmine variety underlying long-term shade (Deng et al. 2012a, b). The rapid protein accumulation reflects the efficient metabolic apparatus of the cultivar in response to shading (Sanmartin et al. 2006). The present results of protein demonstrated again that the jasmine species can acclimate to shaded environment and responded positively to limited irradiance.

MDA content

During ST shading, MDA contents of unshaded plants were significantly ($P < 0.01$ or 0.05) higher than the shaded plants. The highest amounts of all experienced plants were observed in the leaves sampled at 4 HOS (i.e., 12:00), and the values were negatively correlated with the degrees of limited irradiance (Fig. 1c). Similar change was observed in the plants undergone MT shading. At most time points (1, 2, 4 and 5 DOS), the MDA contents in

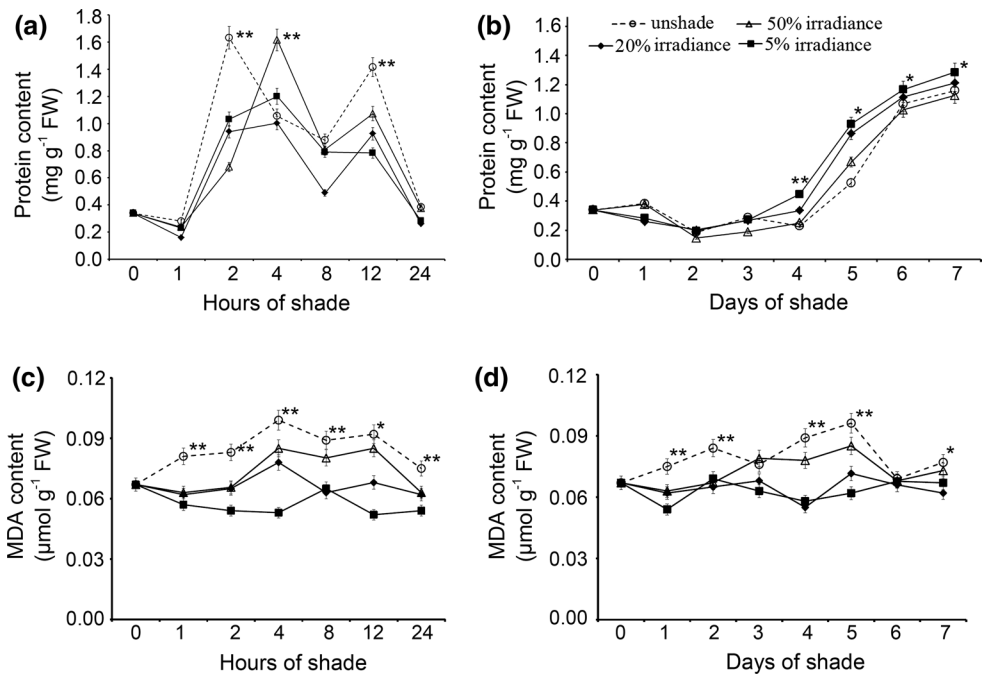

Fig. 1 Effect of full sunlight or various levels of irradiance on jasmine leaf protein and malondialdehyde (MDA) contents during **a, c** 24 h and **b, d** 7 days of shade. Means and standard errors based on three replications. "single asterisk" and "double asterisk" indicate significant differences between the highest and the second high values at the same time point at $P < 0.05$ and 0.01, respectively, as based on the Duncan's multiple range test

unshaded plants were significantly ($P < 0.01$) higher than those shaded ones (Fig. 1d). Simultaneously, the plants under the moderate (20% irradiance) and severe (5% irradiance) levels of shade always contained the fewest MDA. These results were similar to the previous study including both double-petal and multi-petal jasmine varieties underlying a long-term shade (Deng et al. 2012a). MDA is a product of membrane lipid peroxidation, and its content can be used as an indicator of the degree of cellular membrane lipid peroxidation responded to stress (Sharma and Dubey 2007). In the present study, the unshaded leaves containing more MDA suggests that the plants might be suffering oxidative stress under full sun light (especially at solar noon), yet shade could alleviate the degree of damage by reducing the irradiance.

SOD activity

Under ST shading, SOD activity showed almost no change in all sampled plants during the initial 2 HOS (Fig. 2a). However, SOD activity increased quickly from 2 to 4 HOS and maintained at a high level until 24 HOS (Fig. 2a). From midday then on, different degrees of shading showed different effects on SOD activity. For example, at 4 HOS, the SOD activities of plants under varied restricted irradiances were significantly ($P < 0.01$) higher than those unshaded plants (rank order: 5% irradiance > 20% irradiance > 50% irradiance) (Fig. 2a). However, at 12 HOS (i.e., 20:00), the values of 5 and 20% irradiance plants were almost the same, and both of them were significantly ($P < 0.01$) higher than that of weak shaded (50% irradiance) and unshaded plants

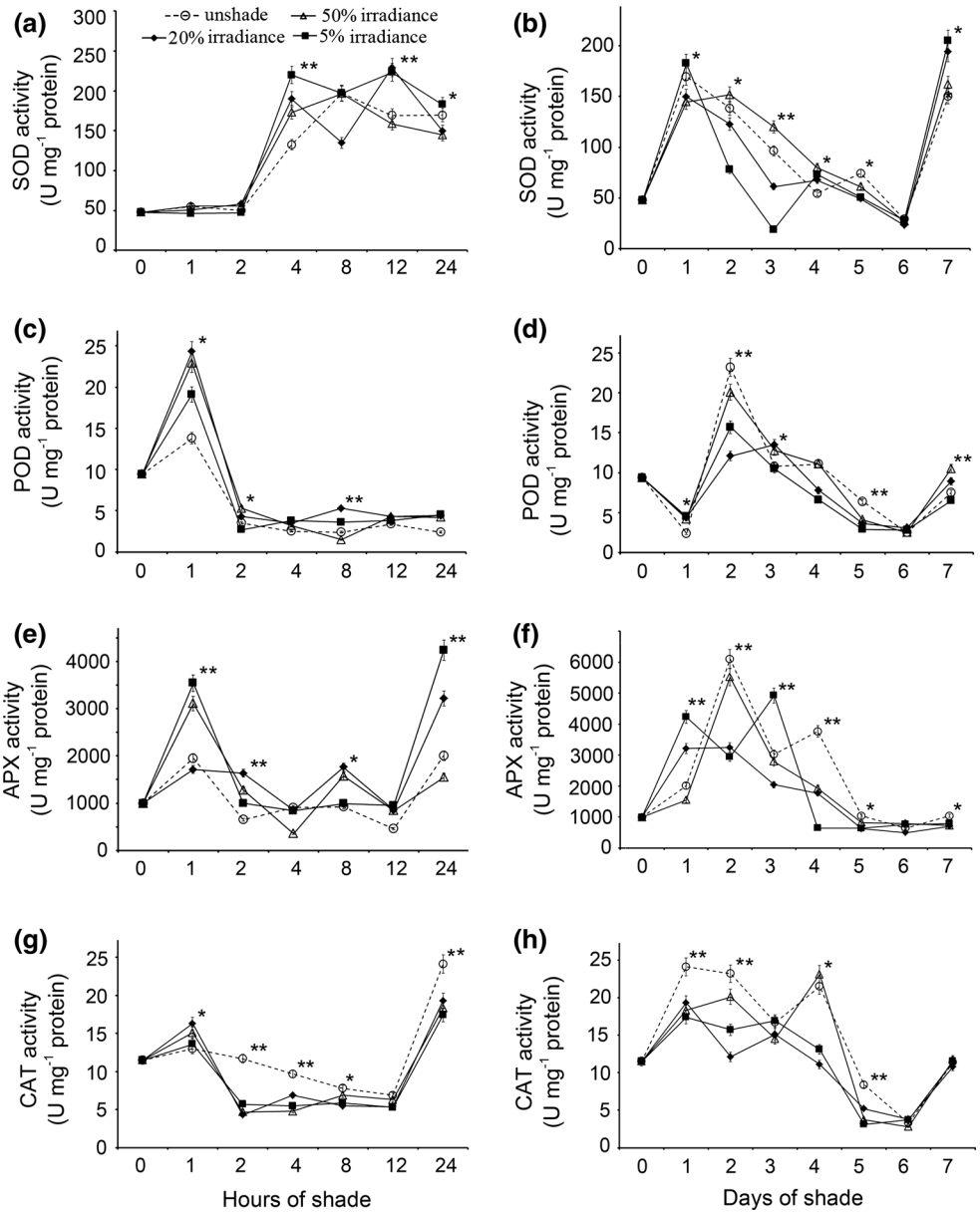

Fig. 2 Effect of full sunlight or various levels of irradiance on **a, b** SOD activity, **c, d** POD activity, **e, f** APX activity and **g, h** CAT activity in jasmine leaves during 24 h (left column) and 7 days (right column) of shade. Means and standard errors based on three replications. "single asterisk" and "double asterisk" indicate significant differences between the highest and the second high values at the same time point at $P < 0.05$ and 0.01, respectively, as based on the Duncan's multiple range test

(Fig. 2a). At the same time, SOD activity in unshaded plants was lower than the shaded plants. This tendency is almost the same as the change of protein content (Fig. 1a). Therefore, it can be speculated that the plants could be suffering photoinhibition under full sunshine irradiance at sunny noon (Guo et al. 2015). On the whole, the SOD activity of unshaded plants was significantly ($P < 0.01$) lower than that of shaded plants (Fig. 2a). This means SOD was induced by ST shading.

Under MT shading, the values increased rapidly during the initial DOS, decreased until 6 DOS, and then increased dramatically again by 7 DOS (Fig. 2b). After 2 and 3 DOS, the SOD activity in severely shaded plants was significantly ($P < 0.01$) lower than that of other plants. However, all samples showed similar results at 6 DOS (Fig. 2b). On the whole, SOD activity was induced by shading, in accordance with another double-petal jasmine variety reported previously (Deng et al. 2012a). According to elevated SOD activity under long-term shade, jasmine was reported to be sensitive to photoinhibition under full sunlight (Favaretto et al. 2011; Deng et al. 2012a). Under ST shade, the plants could be suffering from photoinhibition at 4 HOS (12:00), indicated by the lowest SOD activity in unshaded plants comparing with other shaded ones (Fig. 2a). Therefore, full sunlight may be harmful to jasmine photosynthesis at noon even during a short period, and shade was beneficial to SOD biosynthesis. SOD can catalyze the dismutation of O_2^- to O_2 and H_2O_2, and excessive H_2O_2 can be regulated by APX and CAT (Moller et al. 2007). Therefore, shade was necessary to protect jasmine plant growth under the present conditions. However, severe shade negatively affected SOD biosynthesis with increasing duration. More than 24 h of severe shade should be avoided with respect to maintaining a high SOD activity.

POD activity

POD activity showed an almost opposite response compared to that of SOD. Under ST shade, the value increased approximately 2.5 times within the first HOS, decreased rapidly to 1/5 at 2 HOS, and then was maintained at a nearly stable level until the end (Fig. 2c). At 24 HOS, the POD activity of unshaded plants was significantly ($P < 0.01$) lower than that of shaded plants (Fig. 2c). On the whole, POD activity was enhanced by ST treatment. However, it showed a different change during MT shade. POD activity decreased within the 1st but then increased dramatically by the 2nd d; thereafter, it decreased until 6 DOS but increased again subsequently (Fig. 2d).

On the whole, POD showed an opposite response compared with that of SOD under ST shade. However, with increasing shade duration, these two enzymes showed nearly the same tendency. POD is considered both a protective and a defensive enzyme that simultaneously catalyzes the oxidation of a range of phenolic compounds by the consumption of H_2O_2 and the biosynthesis of lignin and suberin (Ingham et al. 1998). The present results showed that jasmine plants can biosynthesize POD immediately under short-term shaded conditions, which was used quickly together with SOD to protect the plants during a relatively longer shading period. Under 28 and 35 days of long-term shade, POD activity in shaded plants was significantly higher than those unshaded plants (Deng et al. 2012a). However, the present plants did not exhibit such a regular pattern under neither ST nor MT shade treatments. Therefore, the role of POD during short periods of shade might be less important than that during long periods of shade.

APX activity

Under ST shading, the APX activity of shaded plants was higher than that of unshaded plants and increased with increasing degrees of shade (Fig. 2e). For example, after 1, 8 and 24 HOS, the APX values in plants under 5% irradiance were always higher than those of the other treatments, and the difference was significant ($P < 0.01$) (Fig. 2e). However, with increasing duration of the shading treatment, the APX activities were opposite. Under severe shade condition, it was enhanced at 1 and 3 days of shade (DOS), reduced at 2 and 4 DOS, and changed slightly at 5, 6 and 7 DOS (Fig. 2f). On the whole, APX activity showed an increase under ST shading and an irregular change under MT shading, without an obvious decrease as observed for SOD and POD. The APX activity in jasmine plants has been shown to be irregular during 35 days of long-term shading, including double-petal and multi-petal varieties (Deng et al. 2012a). Therefore, APX might be influenced less than other antioxidant enzymes by varying light regimes. APX can regulate excessive H_2O_2 under stress conditions (Moller et al. 2007). An irregular change in APX means it might not be the main enzyme to remove ROS in jasmine plants under shading.

CAT activity

CAT activity was enhanced by different degrees of shade during the 1st HOS. However, the value of shaded plants was significantly lower than that of unshaded plants ($P < 0.05$ or 0.01) from 2 to 24 HOS (Fig. 2g). This tendency was maintained through 6 DOS, with the CAT activity of unshaded plants being higher than that of shaded plants at most time points. For example, at 1, 2 and 5 DOS, the activities of CAT in unshaded plants were significantly ($P < 0.01$) higher than that of shaded plants (Fig. 2h). Therefore, CAT was generally reduced by both the ST and the MT shading. Similar results were observed previously in the double-petal and multi-petal jasmine plants under 35 days of long-term

shading (Deng et al. 2012a). CAT can regulate excessive H_2O_2 under stress conditions together with APX (Moller et al. 2007). Therefore, the relatively lower CAT activity of shaded plants means CAT might be used as one of the main enzymes to remove excess H_2O_2 under shaded environments.

Except APX, the activities of SOD, POD and CAT exhibited an obvious increase at 7 DOS (Fig. 2b, d, h). This might be mainly resulted by the plant physiological status. After 7 days of treatment, the plants grew much vigorously. The axillary buds germinated, and some terminal buds initiated reproductive development. Thus, metabolic level increased and the enzymes activities were enhanced in the plants. In addition, the varying environmental conditions (especially the intensity of sunshine illumination) influenced the plant's metabolic level and might change the enzyme activities.

In conclusion, the protein and MDA contents as well as activity profiles of these four enzymes were significantly influenced by the degree and duration of shade. But the antioxidant enzymes responded differently. Under ST shade, SOD activity increased after 2 h of treating and kept on a high level thereafter, but POD, APX and CAT activities increased mainly during the first hour and then deceased. Under MT shade, the activities of SOD, APX and CAT increased rapidly during the first day and then decreased (SOD), increased (APX) or changed slightly (CAT). However, POD activity was nearly opposite, which decreased during the initial day and increased subsequently. Nevertheless, the rapid changes in protein and antioxidants reflect the efficient metabolic apparatus of jasmine plants in response to restricted irradiance. Shade was necessary to protect jasmine plants against full sun light, especially for avoiding photoinhibition at solar noon. However, severe shade (5% irradiance) should only be suggested for a few hours from 10:00 to 16:00 on sunny days. If the shade lasted for 3–7 days, 20–50% of natural irradiance is more reasonable than the 5% irradiance.

Author contribution statement Yanming Deng designed this study. Xinping Jia, Xiaobo Sun and Lijian Liang conducted the experiment. Yanming Deng and Jiale Su did the analysis. Yanming Deng wrote the manuscript. All authors read the final manuscript and approved the submission.

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