

Physiological Characteristics of Two Rose Cultivars (*Rosa hybrida* L.) under Different Levels of Shading in Greenhouse Conditions

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In many parts of Iran high light intensities during summer can induce stress in cut flower production under open field and greenhouse conditions. Despite roses prefer sunny places for optimum growth, but in practice some levels of rough shading is applied in greenhouse production to improve the quality of cut flowers. So, it is useful to find the light intensities under which different rose cultivars produce optimum yield with acceptable market quality. In present study, different light intensities were applied for two rose cultivars of 'Red One' and 'Gulmira', by shading levels of 1200 (without shading as control), 640, 520 and 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$, using green plastic nets. The result showed that leaf protein concentration, peroxidase activity, petal anthocyanin concentration and carbohydrate levels were significantly different among shade treatments, as the highest protein concentration was observed in 640 $\mu\text{molm}^{-2} \text{s}^{-1}$. The highest concentration of anthocyanin of petals was observed in 'Red One' under 520 $\mu\text{molm}^{-2} \text{s}^{-1}$. Furthermore, in 'Gulmira' the highest amount of anthocynin was in 520 $\mu\text{mol m}^{-2} \text{s}^{-1}$, but this was not statistically significant compared to other shading treatments. The highest amount of leaf carbohydrate was in 520 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The results indicate that shading up to a light intensity of 520 $\mu\text{mol m}^{-2} \text{s}^{-1}$ is beneficial for quality improvement of these rose cultivars when light intensity is high.

Abstract

Keywords: Anthocyanin, Catalase, Light intensity, Peroxidase, Physiological traits, Quality, Rose.

INTRODUCTION

Roses are generally on the top in the world fresh cut flowers market. Rose production for cut flowers is mainly under greenhouse conditions. Rose cut flowers play important role in economy aspects in agriculture, so optimizing growth conditions for better yield and quality is very importance.

Light is one of the most important climatic factors in rose production. However, in tropical, sub-tropical and some temperate regions, high light intensities during summer could be stressful in cut flower production under open field as well as greenhouse conditions. In the most regions of Iran such as Pakdasht, which are involved in cut rose production, high light intensity from April till November is rather a problem inducing high light stress and heat stress. The way and the degree for shading to reduce light level is a big challenge for rose growers. This is quite different from European countries in which complementary light is used to improve the quality of rose production (Bredmose, 1993). In practice, for optimum growth and quality, high light intensity during summer is a problem in countries like Iran. Nevertheless, numerous studies have been conducted in different countries to determine the optimum shading level regarding the yield, quality and physiological behavior of different plant species and cultivars (Villegas *et al.*, 2006; Jeong and Pasian, 2007; Cermeneo *et al.*, 2001; Duriyaprapan and Britten, 1982; Ganele vin and Zieslin, 2001; Hlatshwayo and Wahome, 2010).

Many studies focus on the effect of light on plant physiological behavior, as light is the main environmental factor that affects pigment accumulation in flowers. Chlorophyll biosynthesis and degradation is actively regulated by light intensities (Taiz and Zieger, 2002; Estaji *et al.*, 2011). For maximum photon absorption, plant changes its chlorophyll content under different environmental conditions. Light intensity affects phenolic metabolism including anthocyanins biosynthesis (Taiz and Zieger, 2002). In some apple cultivars considerable amounts of anthocyanin is produced in light-side of fruits (Merzlyak and Chivkunova, 2000); however, shade-side leaves may have higher chlorophyll content (Taiz and Zieger, 2002). Increase or reduction in light intensity may change carboxylation or respiration rates. In roses carbohydrate levels were reduced with low light intensity under control conditions (van Doorn and Vojinovic, 1996). Similarly, in another study the content of sucrose, glucose and fructose in petal cells of rose plant were decreased at low light intensity of $15 \mu\text{mol m}^{-2}\text{s}^{-1}$ (van Staden *et al.*, 1981). Nevertheless, the plant carbohydrate biosynthesis is highly dependent on adequate light intensity.

There may be also a high correlation between light intensity and relative water content (RWC) (Souri *et al.*, 2009). It has been reported that the RWC in rice tissues decreased linearly with increasing light intensity (Zhou *et al.*, 2007). In many cases, high light intensities cannot increase the rate of photosynthesis, but frequently results in leaf sunburn or other related disorders (Dole and Wilkins, 1999). Excessive light normally increases the leaf thickness and the number of leaves; however, leaf area may be reduced due to low rate of cell division (Taiz and Zieger, 2002). Many morphophysiological characteristics of plants including leaf orientation may also change with high light intensities. It is likely that excessive light may degrade photosynthesis-related pigments or lead to an increase in oxygen reactive species (ROS) in thylakoid membranes by interruptions in electron transfer chains, resulting in plants to be faced with secondary oxidative stress (Faust, 2004). Therefore, this study was conducted to determine physiological behavior and quality responses of rose flowers to different shading levels under greenhouse conditions.

MATERIALS AND METHODS

This study was conducted in a commercial rose production greenhouse under hydroponics system in Pakdasht- Iran during 2011. The experimental design was arranged as factorial based on randomized complete blocks with four replications. Each replicate consisted of 10 rose plants. Two cultivars of rose namely 'Red One' and 'Gulmira' representing red and white colors were used. These cultivars were subjected to different shading treatments from 21th June until end of

September. Shadings were applied using green plastic nets with different mesh (densities), to induce different light intensity treatments. Different light intensities were applied including 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (as control without shading), 640, 520, and 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Light intensities were measured and controlled using a LUX meter which the records were calculated for more scientific unit of $\mu\text{mol m}^{-2} \text{s}^{-1}$. Different plant traits were measured including: content of chlorophyll, anthocyanin, protein and carbohydrate, relative water content (RWC), and activity of catalase and peroxidase enzymes.

Dimethyl sulfoxide solvent (DMSO) was used for leaf chlorophyll extraction in dark, and absorbance of extracts was read spectrophotometrically (Lambada 25, Perkin Elmer, USA) at 645 and 663 nm for chlorophyll a (mg/g FW) and chlorophyll b (mg/g FW). Carotenoid content (mg/g FW) were also calculated from the extract by measuring its absorbance at 470 nm and 510 nm using following equations:

$$C \text{ Chla (mg/g FW)} = 12.7(A_{663}) - 2.69(A_{645}) \times V / (1000 \times W)$$

$$C \text{ Chlb (mg/g FW)} = 22.9(A_{645}) - 4.68(A_{663}) \times V / (1000 \times W)$$

$$C \text{ TChl (mg/g FW)} = 20.2(A_{645}) + 8.02(A_{663}) \times V / (1000 \times W)$$

$$C \text{ Carotenoid (mg/g FW)} = 7.6(A_{470}) - 1.49(A_{510}) \times V / (1000 \times W)$$

For determination of anthocyanins, 0.1 g fresh weight of petals was finely grinded in a mixture of 10 mL acidified methanol (methylic alcohol and chloridric acid in a ratio of 99:1 (v/v))(1% HCl). The solution was centrifuged and the supernatants were kept overnight in darkness. Absorption was determined spectrophotometrically at 550 nm (Lambada 25, Perkin Elmer, USA). The concentration of anthocyanins ($\mu\text{mol g}^{-1}$ FW) was calculated using extinction coefficient ($\epsilon=33000 \text{ cm}^2\text{mol}^{-1}$) and the following $A = \epsilon bc$ formula, where A is sample absorption, b is cuvette width and c is the solution concentration.

Anthrone reagent was used for extraction of soluble carbohydrates. For this purpose 0.5 g fresh sample of fully developed leaves was crushed in porcelain mortars with 5 ml ethanol 95%. The supernatant was separated and the remaining rewashed by 5 ml ethanol 70%, and added to the last solution. The extract was centrifuged for 15 min at 4500 rpm. The supernatant was used for carbohydrate measurement. Three ml of anthrone solution (150 mg pure anthrone + 100 ml sulfuric acid 72%) was added to 100 μl of extract. The mixture transferred to water bath for full reaction until extract got colored. Then, its absorption was determined by spectrophotometer at 625 nm (Lambada 25, Perkin Elmer, USA). The standard solutions were 100.17, 204.19, 301.82, 401.26 and 503.79 mg/l pure glucose. '0' was used as blank.

Relative water content (RWC) was determined as follows: $\text{RWC} = (\text{fresh weight} - \text{dry weight}) / (\text{fully turgid weight} - \text{dry weight})$

To determine the fully turgid weight, leaves were kept in distilled water in darkness at 4°C to minimize respiration losses until they reached a constant weight (full turgor, typically after 24-48 h). Leaf dry weight was recorded after drying at 80 °C for 24 h in an oven (Model 271C, Made in Korea).

Protein content was determined using Bradford methods (Bradford, 1976). For this purpose 100 mg of Coomassie Blue G was dissolved in 50 mL of methanol (95%). The solution was added to 100 mL of 85% H_3PO_4 , and diluted to 1000 mL with distilled water. The solution was transferred into an amber bottle at 4°C. Bovine serum albumin (BSA) was used as standard. Five mg BSA was dissolved in 5 mL of phosphate sodium buffer (50 mM) (pH=7). A convenient standard curve was made using BSA with concentrations of 10, 25, 30, 1000, 40, 50, 60 and 70 μL . Catalase and peroxidase enzymes were measured by method described by Aebi, (1984).

Data were statistically analyzed using SPSS16 software and comparison of means was done at 5% level of LSD test.

RESULTS AND DISCUSSION

The results of analysis of variances are presented in Table 1. The results show that the interaction of light intensity and rose variety was not significant for chlorophyll concentration, leaf soluble carbohydrates and leaf catalase activity, while there was significant difference for leaf protein concentration at 5% and for petal anthocyanin concentration, leaf RWC and peroxidase activity at 1% level.

The highest and lowest leaf chlorophyll concentrations in both cultivars was in 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (the highest shading level) and in control (without shading), respectively (Fig. 1). Cultivar 'Red One' showed higher chlorophyll concentration compared to Gulmira. With increasing shading levels (1200 to 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$) all three parts of chlorophyll (Chl a, Chl b, and total chlorophyll) were constantly increased (Fig. 1).

In this study, the effects of shading, cultivar, and their interactions on flower anthocyanin concentrations were significant. The highest and the lowest anthocyanin concentration in 'Red One' were observed in (520 $\mu\text{mol m}^{-2} \text{s}^{-1}$), and in the highest shading level (240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity) treatments, respectively (Fig. 2). 'Red One' showed higher anthocyanin concentration than 'Gulmira' cultivar. However, in 'Gulmira' anthocyanin concentration did not show significant difference under different shading levels (Fig. 2).

Carbohydrate concentrations showed significant differences between two rose cultivars and under shading treatments. The highest leaf carbohydrate concentration in 'Gulmira' and 'Red One' were obtained under 520 and 640 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, respectively. The lowest carbohydrate concentration for both cultivars was observed under 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity. However, the average amount of carbohydrates in 'Gulmira' was more than 'Red One' (Fig. 3). In 'Red One' cul-

Table 1. The results of analysis of variances for various traits in this study.

S.o.V	df	Chlorophyll con.	Petal anthocyanin con.	Soluble carbohydrate	RWC	Soluble protein	Activity of catalase	Activity of peroxidase
Replication (R)	3	0.002 ^{ns}	1134.43 ^{ns}	3798727.02 ^{ns}	221.91 ^{ns}	4.87 ^{ns}	0.0000062 ^{ns}	2.08 ^{ns}
Light intensity (L)	3	6.43 ^{**}	17903.31 ^{**}	15287360.99 [*]	56.61 ^{ns}	60.24 ^{**}	0.000013 ^{ns}	9.7 ^{**}
R×L	9	0.031 ^{ns}	2931.97 ^{ns}	8385879.41 ^{ns}	76.45 ^{ns}	8.06 ^{ns}	0.000014 ^{ns}	1.45 ^{ns}
Variety (V)	1	0.22 ^{ns}	7826256.72 ^{**}	39014919.45 ^{**}	27.26 ^{ns}	616.62 ^{**}	0.00063 ^{**}	0 ^{ns}
L×V	3	0.38 ^{ns}	17859.93 ^{**}	11228715.40 ^{ns}	654.67 ^{**}	26.08 [*]	0.0000035 ^{ns}	0.0067 ^{ns}
Error	12	1.72	2391.23	3735199.8	104.99	4.52	0.000015	1.25
CV (%)		6.14	9.77	11.11	14.04	19.75	61.29	37.71

ns, * and ** mean no significant difference, significant difference at 5% and 1%, respectively.

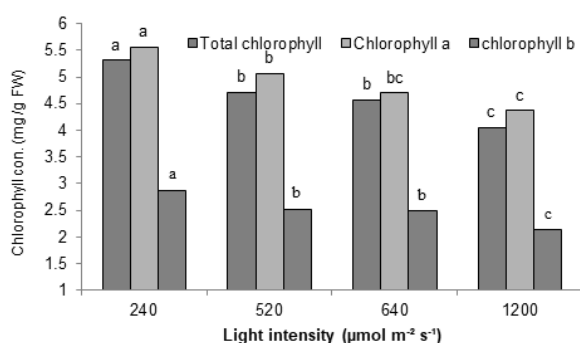


Fig. 1. Chlorophyll concentrations in two rose cultivars under different shading levels. Means with the same letter are not significantly different at 5% level of LSD test.

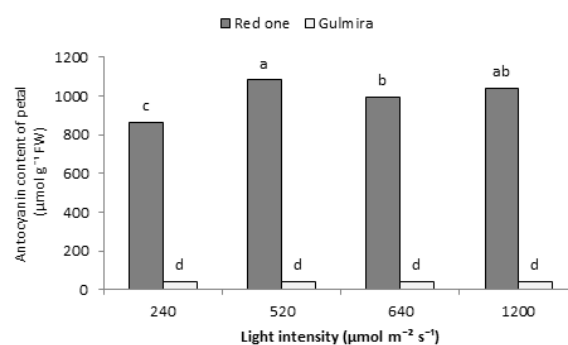


Fig. 2. The concentration of total anthocyanin in two rose cultivars under different shading levels. Means with the same letter are not significantly different at 5% level of LSD test.

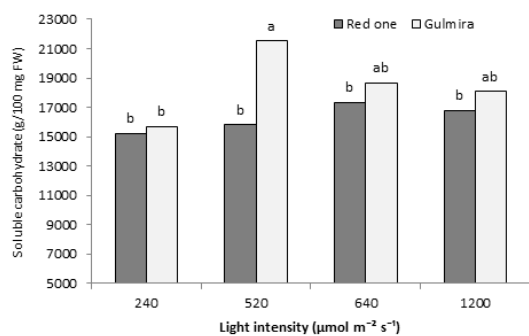


Fig. 3. Concentration of soluble carbohydrates under different shading levels in two rose cultivars. Means with the same letter are not significantly different at 5% level of LSD test.

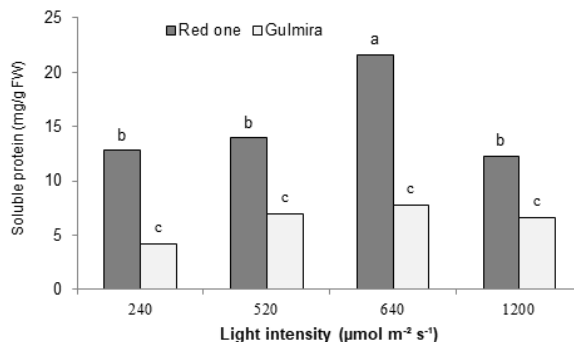


Fig. 4. Soluble protein concentrations in two cultivars of rose under different shading levels. Means with the same letter are not significantly different at 5% level of LSD test.

tivar there was no significant difference for carbohydrate contents among light intensities (Fig. 3).

Leaf relative water content (RWC) showed significant differences at 1% level of LSD test for interaction effect of shading and cultivar. Different shading treatments had no significant effect on RWC; however, the highest RWC was observed in plants under 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatments.

In this study, protein concentration and the activity of catalase and peroxidase enzymes were significantly affected by shading treatments (Figs. 4, 5 and 6). The highest and the lowest amount of protein concentration in ‘Red One’ cultivar were observed under 640 and 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, respectively. In ‘Gulmira’ there shading levels had no significant effect on protein concentration (Fig. 4). In general, the average concentration of protein in ‘Red One’ was higher than ‘Gulmira’ (Fig. 4).

The catalase activity was significantly different between two cultivars, and it was much higher in ‘Gulmira’ rather than ‘Red One’. Different shading levels had no significant effect on the activity of this enzyme (Fig. 5). Peroxidase activity in both cultivars was reduced by increasing shading levels; as the highest peroxidase activity was in control plants without shading (highest light intensity) (Fig. 6).

Results showed that in both rose cultivars, chlorophyll content was increased under higher shading levels. Chlorophylls are the pigments responsible for photosynthesis and CO_2 fixation, and those plants under higher light intensity generally produce more carbohydrates (Hosseini *et al.*, 2014). Chlorophyll concentration of leaves is very sensitive to many environmental factors including light intensity, temperature and nutrients availability (Souri and Römheld, 2009); however, under different environmental conditions, chlorophyll changes and adapt for highest photon absorption. Direct sunlight and high light intensity can damage chlorophylls (Merzlyak and

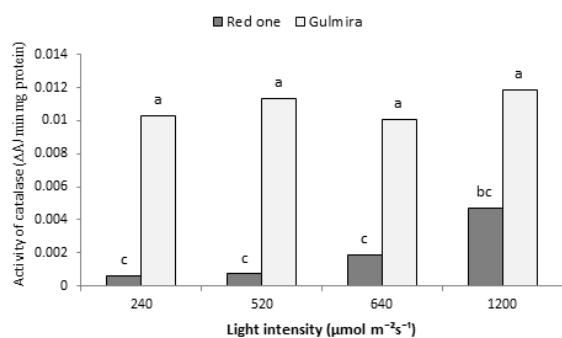


Fig. 5. The activity of catalase enzyme in two rose cultivars under different shading levels. Means with the same letter are not significantly different at 5% level of LSD test.

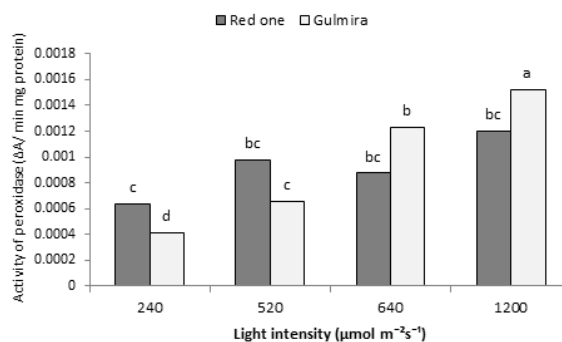


Fig. 6. Activity of peroxidase in two cultivars of rose under different shading levels. Means with the same letter are not significantly different at 5% level of LSD test.

Chivkunova, 2000) as well as other photosynthetic pigments (Faust, 2004). The synthesis of chlorophyll is inhibited under high light intensities (Taiz and Zeiger, 2002), and the degradation of chlorophyll is highly active under high light intensities; therefore chlorophyll content reaches its optimum level under some shade conditions (Brand, 1997). This indicates that some levels of shading might be beneficial in practice. In begonia, the highest chlorophyll content was observed in plants under 76% shading (Jeong and Pasian, 2007), and plants grown in sunlight had less chlorophyll content (Hamerlynck *et al.*, 2000). Similar results were obtained in present study. This is probably due to the fact that plants in shade receive less light for photosynthesis (Hosseini *et al.*, 2014), therefore they increase the leaf area and change photosynthetic pigments to compensate the light shortages (Hamerlynck *et al.*, 2000).

Similarly, accumulation of anthocyanin as an important pigment is influenced by different environmental variables including light conditions (Merzlyak and Chivkunova, 2000). Anthocyanins have high stability to light and they are able to absorb UV, green and blue lights, as they have a role as light protective for other cell components especially chlorophylls. Therefore, their accumulation could be a protective mechanism against harmful light effects (Oren-Shamir and Levi-Nissim, 1997). Under high light intensities, some changes in pigment metabolism and compositions may occur (Merzlyak and Chivkunova, 2000), which mainly act to protect chlorophyll from harmful effects of higher light intensities (Hamerlynck *et al.*, 2000). In 'Baccara' rose, low light intensity reduced plant sugar levels, leading to less pigment synthesis, a phenomena known as "Bluing" in rose cultivation (Biran and Halevy, 1974). Similarly in 'Ehigasa' cultivar of floribunda roses, dark condition resulted in colorless petals, but with increasing of light period and intensity, anthocyanin production was increased (Maekawa *et al.*, 1980).

In present study, leaf carbohydrate concentration was higher in 520 and 640 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in 'Gulmira' and 'Red One', respectively. Carbohydrate accumulation is a key feature reflecting species differences for growth and survival under different environmental stresses (Farhadi *et al.*, 2013). New shoot production is reduced under higher shading levels probably due to assimilate limitations (Taiz and Zaiger, 2002; Souri and Roemheld, 2009). Therefore, the low level of carbohydrates in plants grown under low light intensities and control conditions such as greenhouse and growth chamber is well known (Souri *et al.*, 2009). The content of sucrose, glucose, and fructose in petal cells of rose plant are reduced under low light conditions (15 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (van Doorn and Vojinovic, 1996). In some plants, such phenomena frequently happening in leaves, so improvement of light condition may increase carbohydrate levels leading to better growth under stress conditions (Xu *et al.*, 2004). In *Equisetum arvense* L. it was shown that after 13 days of shading, plants still had high amount of sucrose in their shoots and rhizome, indicating no changes in carbohydrate levels by shading (Sakamaki and Ino, 2002).

Light intensity has an inevitable role on leaf relative water content (RWC); and in present study the highest RWC was observed in plants under 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment. It has been shown that the amount of RWC in light-side leaves may be more than shade-side leaves (Mc Cain, 1995). Both light and temperature are effective on water status of plant (Souri *et al.*, 2009) and consequently on product quality. Similar to other plant species, roses could lose more water in light rather than shade conditions. Changes in photosynthesis rates can also affect plant water relations. Root system and water uptake characteristics are effective on leaf RWC and transpiration rate (Souri *et al.*, 2009). Any changes in gradient of water potential from root to leaves and on stomata openings are important factors in this regard. Therefore, in shading treatment of 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ reduction in transpiration has led to higher RWC records.

Protein concentration in both rose cultivars was highest under moderate light intensity (640 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and the lowest protein was observed in 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment. It was shown that light increases the concentration of some proteins such as 13, 18, 40 and 67 KD while reducing some others (Kallies *et al.*, 1992). Protein degradation and inactivation of some enzymes are actively

occurring under high light intensities; however, plant species may have various different mechanisms for protein/carbohydrate ratio adjustment under different light levels (Taiz and Zeiger, 2002).

Shading levels had no significant effect on catalase activity; however, peroxidase activity was increased in both cultivars with higher light intensities (Fig. 6). It seems that at higher light intensities, plant increases the antioxidant levels for scavenging of ROS molecules (Taiz and Zeiger, 2002). On the other hand, in response to increased ROS production, the expression of genes encoding antioxidant proteins is generally reduced (Malek Ahmadi *et al.*, 2005). Under normal conditions, reactive oxygen species (ROS) are produced at low levels in plant cells by various metabolic processes. Oxidative stress is induced when ROS production exceeds the cell's ability to detoxify them. Overproduction of ROS damages cellular components including chlorophylls, and membrane lipids, leading to decline in physiological function and cell death (Taiz and Zeiger, 2002). Under different environmental stresses, metabolic disturbances in plant cells lead to ROS production particularly by light excitement (Taiz and Zeiger, 2002). Therefore, high light intensity stress can result in ROS production in photochemical reaction center of plant cells (Wilson *et al.*, 2008).

In plants, extra light may be converted to heat under normal light condition (Wilson *et al.*, 2008). In higher light intensities this strategy cannot protect the plant and light damages the photosystem II resulting in ROS production. During all physiological processes in plant tissues, the poisonous hydrogen peroxide (H_2O_2) may be produced. Catalase and peroxidase are the main enzymes involved in inactivation of ROS (Taiz Zeiger, 2002). The activity of peroxidase enzyme is an index for physiological changes and metabolic activity inside the plant. In present study catalase is probably inactivated by higher light intensity in rose leaves. In green or etiolated leaves of rye, catalase was inactivated by blue light (Shang and Feierabend, 1999). However, red light at low light intensity was not effective in enzyme inactivation. The highest inactivation was in green leaves with higher photon influxes (Shang and Feierabend, 1999). Catalase and peroxidase activity in short period of light treatments were reduced in radish, while the H_2O_2 contents was increased. Light and temperature are key elements in radish antioxidant activity (Zhang *et al.*, 2009). In present study, increasing leaf peroxidase activity with light intensities in both rose cultivars probably plays role in scavenging of ROS as an effective antioxidants. It was reported that peroxidase activity after two days of irradiation was increased (Liu *et al.*, 1996). The catalase activity was also higher in light rather than shade plants (Liu *et al.*, 1996). Under continuous light, the activity of ascorbate peroxidase was increased in radish roots two days after treatment. An overall higher enzyme activity was also reported in H_2O_2 scavenging system (Liu *et al.*, 1996). Nevertheless, peroxidase activity is depended on irradiation and light spectra composition, because the enzyme has especial sensitivity to red and blue spectra of light (Zhang *et al.*, 2009).

In conclusion, the results of present study showed that physiological behavior and antioxidant activity of both rose cultivars were affected by different shading levels. According to the results, the highest amounts of quality related traits (carbohydrate and anthocyanins content) were observed in $520 \mu\text{mol m}^{-2} \text{s}^{-1}$ treatment in both rose cultivars. Higher light intensities resulted in higher peroxidase activity probably to protect plants against possible ROS, while catalase enzyme was mainly inactivated under higher light intensities. Therefore, for growing of these two rose cultivars under greenhouse conditions, light intensity of $520 \mu\text{mol m}^{-2} \text{s}^{-1}$ is probably the most effective treatment for cut flower production with improved quality.

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