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Red and Blue Lights Significantly Affect Photosynthetic Properties and Ultrastructure of Mesophyll Cells in Senescing Grape Leaves

WANG Shuai, WANG Xiaodi, SHI Xiangbin, WANG Baoliang, ZHENG Xiaocui, WANG Haibo *, and LIU Fengzhi *

Fruit Research Institute, Chinese Academy of Agricultural Sciences/Key Laboratory of Biology and Genetic Improvement of Horticultural Crops(Germplasm Resources Utilization), Ministry of Agriculture, Xingcheng, Liaoning 125100, China

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Abstract

Light quality significantly affects photosynthetic efficiency in plants. The mechanisms for how light quality affects photosynthesis in grape is poorly understood. Therefore, to investigate the effects of different light qualities on chloroplast ultrastructure and photosynthesis efficiency, two grape cultivars 'Italia' (slower speed of leaf senescence) and 'Centennial Seedless' (faster speed of leaf senescence) grown under protected and delayed conditions were used. The three treatments, replicated three times, were control (no supplemental lighting), red light and blue light. Chlorophyll content, net photosynthetic rate, and the ratio of F_v/F_m significantly increased in red light relative to the control. The opposite trend was observed in blue light in the early phase of leaf senescence. At later stages, physiological indexes were gradually higher than that of control, resulting in a delay in leaf senescence. Compared to the control, red and blue light both significantly increased the chlorophyll a/b ratio. Electron microscopy showed that blue light caused severe damage to the fine structure of chloroplasts at early stages of leaf senescence, but effects at later stages of leaf senescence became less severe compared to the control. The degradation of chloroplast ultrastructure was apparently delayed in red light throughout the experimental timeframe compared to other treatments. In this experiment, 'Italia' showed higher chlorophyll content, net photosynthetic rate, ratios of F_v/F_m, chlorophyll a/b and better preserved chloroplast ultrastructure relative to 'Centennial Seedless', resulting in a slower rate of leaf senescence.

Keywords: grape; leaf senescence; red and blue light; chloroplast ultrastructure; electron microscopy

1. Introduction

Leaf senescence, as the last stage of leaf development, is often regarded as a type of programmed cell death (PCD) (van Doorn and Woltering, 2004). The senescence process is accompanied by degradation of chlorophyll and protein, a decline in photosynthesis, remobilization of nutrients to developing or storage organs and oxidative degradation of lipids (Hameed et al., 2013; Nath et al., 2013; Jakhar and Mukherjee, 2014). One of the most interesting events occurring at the onset of leaf senescence is the dismantling of the photosynthetic apparatus within chloroplasts (Miersch et al., 2000; Wingler et al., 2004). Senescence can be characterized by specific cell ultrastructural changes (Inada et al., 1998). Chloroplast breakdown within deteriorating thylakoids is one of the earliest and most conspicuous changes in cell structure as senescence proceeds, while the nucleus and mitochondria are the last to be affected during the senescence process (Smart, 1994; Gan and Amasino, 1997; Prakash et al., 2001; Lee et al., 2004). The mitochondria can be seen flanking the chloroplast and retaining ultrastructural integrity until late senescence (Miller et al., 1999; Evans et al., 2010). Electron microscopy reveals that chloroplasts in senescent leaves differentiate into gerontoplasts, which contain stack-less thylakoid membranes and increased accumulation of stroma lipid–protein substance in plastoglobules (Tevini and Steinmüller, 1985; Kratsch and Wise, 2000; Kusaba et al., 2007; Wada et al., 2009). Plastoglobules are major characteristics of gerontoplasts in senescent leaves. The dismantling of thylakoid membranes is accompanied with decreased photosynthesis in senescent chloroplasts. This can be attributed partly to significant loss of photosynthetic electron transport capability of photosystem I (PSI) found in stroma thylakoids and photosystemII (PSII) found in grana thylakoids during

* Corresponding author. Tel.: +86 13591963796.
E-mail addresses: haibo8316@163.com; liufengzhi6699@126.com

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senescence (Goodchild and Park, 1971; Guiamét et al., 2002). Therefore, loss of photochemical efficiency (F_v/F_m), representing photosynthetic capacity and the structural and functional integrity of PSII, can be used as to measure senescence (Oh et al., 1996).

Many physiological, biochemical, molecular and ultrastructural studies have shown that leaf senescence is regulated by a complex array of endogenous factors such as age and hormones, and environmental factors such as light, drought, extreme temperature, and pathogen infection (Smart, 1994). Light in particular plays a critical role in leaf senescence (Ono et al., 2001). Borrás et al. (2003) proposed that light quality was closely related to leaf senescence. Light quality plays a crucial role in photosynthetic processes, and its energy inevitably modulates the processes of photosynthesis. Furthermore, the quality of light alters the structure and function of chloroplasts in leaves (Kim et al., 1993; Albertsson, 2001). Different blue and red light treatments have been used to analyze the ultrastructure of organelles in plant cells (Pang et al., 1998; Shaver et al., 2008). Although much work on leaf senescence has been performed in plants, no single comprehensive study has been performed to measure the effect of different light spectral quality on photosynthetic properties and ultrastructure of chloroplasts during leaf senescence in different grape species.

Grapes (*Vitis vinifera* L.) are one of the most economically important fruit crops in the world, mainly distributed in north China (Pang et al., 1998). In recent years, with improvement in living standards and market demand, grape cultivation using greenhouses has developed rapidly in many regions. Delayed planting significantly improved the economic benefits of protected cultivation. However, leaf senescence problems seriously affect the maintenance of mature fruit quality and have become the main restrictive factor for the sustainable development of protected grape cultivation. Thus study of factors involved in leaf senescence has theoretical and practical significance for grapes in delayed cultivation.

In the present experiment, we chose two grape varieties of *Vitis vinifera* — 'Italia' and 'Centennial Seedless' — and studied different senescence-associated characteristics in photosynthetic properties and chloroplast ultrastructure in leaves exposed to different light spectral qualities (blue light and red light) at different stages of senescence. The objective of the study was to elucidate the response of leaf senescence to red light and blue light and to determine whether the chloroplasts were representative of the overall senescence symptoms caused by light treatments, providing a theoretical basis for anti-aging technology of grapes under delayed cultivation.

2. Materials and methods

2.1. Plant materials and growth condition

The experiment was conducted in a greenhouse at Institute of Pomology, Chinese Academy of Agricultural Sciences in Xingcheng, Liaoning, China (40°16′N, 120°06′E) from August 2013 to January 2014. The greenhouse was equipped with a heat pump and environmental conditions were maintained with day/night temperature regimes of 27 °C/18 °C, and relative air humidity of 75%–80%. Three-year-old 'Italia' (a late ripening variety whose fruit mature from late September to early October and were completely defoliated period in 2013 from November 28 to December 5, with a slower rate of leaf senescence) and 'Centennial Seedless' (a medium ripening variety whose fruit mature in the middle of August and were completely defoliated in 2013 from November 21 to November 27, with a quicker rate of leaf senescence) were used, with Beta as rootstock. The tree was characterized by a single level dragon trunk with a V-shaped canopy. Plants were alternated in 0.7 m and 2 m wide rows referred to as narrow- and wide-spacing, respectively.

From 1 August 2013 to loss of leaves, red light or blue light (the peak wavelength of red light and blue light were 610 nm and 435 nm) were provided as supplemental lighting, with no supplemental lighting as a control. The 38 w plant growth light was provided by Shanghai Heming Lighting Co. Ltd. There were two lines of plants serving as guard row among different treatments, with 45 plants per treatment. The experiment was designed as a completely randomized block design with three replicates. The lamps were mounted at a height of 3.5 m above the ground (equivalent to 0.4 m above the top of the full-grown canopy) with 2 m spacing between lamps. There were two lamps per lighting treatment and light intensity, determined by the TES-1332A digital illumination meter, was (30 ± 5) μmol · m^{-2} · s^{-1}. The lamps were turned on a half-hour before sunset and turned off at 0:00 a.m. (times were adjusted according to the day of the year and varied between 5 h and 6 h). Experimental treatments with different lights were maintained until abscission.

Representative grape plants were selected as experimental plants, and 20 functional leaves of labeled plants were measured corresponding to intervals of 15 days. All of the experiments were repeated three times; data presented are means of all experiments with every assay per experiment for each variety.

2.2. Chemical analysis

Determination of chlorophyll content: triplicates of fresh leaf material (a mixture of 20 leaves for each date) were extracted in 80% acetone, and the extract was analyzed for absorbance at wavelengths of 645, 663 and 652 nm. The concentrations of chlorophyll a and chlorophyll b were then calculated using the following equations (Arnon, 1949).

$$\text{Chl. a}\left(\text{mg}\cdot\text{g}^{-1}\right) = \left(12.7\times \text{OD}_{663} - 2.69\times \text{OD}_{645}\right)\text{V}/1000\ \text{W};$$

$$\text{Chl. b}\left(\text{mg}\cdot\text{g}^{-1}\right) = \left(22.9\times \text{OD}_{645} - 4.68\times \text{OD}_{663}\right)\text{V}/1000\ \text{W};$$

$$\text{Chl. (a + b)}\left(\text{mg}\cdot\text{g}^{-1}\right) = \text{Chl. a} + \text{Chl. b};$$

$$\text{Chl. a/b}\left(\text{mg}\cdot\text{g}^{-1}\right) = \text{Chl. a}/\text{Chl. b}.$$

Fluorescence measurements for F_v/F_m at room temperature were conducted with a Chlorophyll Fluorescence Imager (CFI) (Technologica, UK) using modulated pulse techniques. Measurements were made on single leaf-discs (diameter = 10 mm) for each treatment that were acclimated to darkness for 30 min prior to measurements. Visible light intensity was set to a low radiation (800 μmol · m^{-2} · s^{-1}) using fluorescent tubes for 30 min. Using both light and dark fluorescence parameters, maximal