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Flower greening in phytoplasma-infected *Hydrangea macrophylla* grown under different shading conditions

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ABSTRACT

To determine the effect of light intensity on flower greening, the Japanese hydrangea phyllody (JHP) phytoplasma-infected hydrangea cultivars 'Midori', 'Libelle', 'Rosea' and 'Madame E. Mouillere' plants were grown under different shade conditions. In the first-year experiment, the results indicate that the flowers of the JHP-phytoplasma-infected hydrangea become green under shaded conditions (70% and 49% sunlight intensities). On the other hand, under full sunlight intensity (100% sunlight intensity), the flowers of 'Midori', 'Rosea', and 'Libelle' plants were blue, pink or white. To calculate the percentage of flower greening, inflorescences of these plants were separated and divided into individual flowers, and classified into four types by green-area ratio, calculated using Adobe Photoshop. Under shading with one sheet of cheesecloth (70% sunlight intensity), the inflorescences of 'Midori', 'Libelle' and 'Madame E. Mouillere' plants were composed of more than 40% completely green flowers ($0.8 \leqq$ green-area ratio), whereas those of 'Rosea' plant had 0% completely green flowers. Under shading with two sheets of cheesecloth (49% sunlight intensity), the inflorescences of 'Midori', 'Libelle' and 'Madame E. Mouillere' plants had more than 75% completely green flowers; 'Rosea' plants had 28%. In the second-year experiment, under full sunlight intensity, 'Midori' plants had four types of flower depending on their green-area ratio, namely, completely blue or pink, pink-green, greenish and completely green flowers. Under shading with two sheets of cheesecloth, 'Midori' plants had more than 90% completely green flowers. The JHP-phytoplasma could not be identified by PCR analysis in flowers with a green-area ratio = 0 (completely blue/pink/white flowers). On the other hand, in flowers with a green-area ratio > 0, the JHP-phytoplasma was detected by PCR analysis. Thus, we conclude that shading enhances flower greening in hydrangea by increasing the JHP-phytoplasma concentration in the flowers.

1. Introduction

Hydrangea macrophylla, a woody genus of Saxifragaceae, has been a popular garden and greenhouse plant since 1789 (McClintock, 1957). This plant is valued for its large brightly colored flowers. Hydrangeas grow well at moderate temperatures (Bailey, 1914). During summer production at northern latitudes (42°–59°N), they grow vigorously under little or no shading (Littlere and Stromme, 1975); however, they also grow well under moderate shading (20–50%) at southern latitudes (32°–42°N) (Shanks and Link, 1951). Hydrangeas prefer partial shading (30%) in areas and seasons with high light intensities (Weiler, 1980), and a definite light minimum is needed for adequate shoot development and flower bud differentiation (Ray, 1946; Struckmeyer, 1950; Littlere and Stromme, 1975).

In 1996, Kanehira et al. reported on a Japanese hydrangea phyllody (JHP) disease of hydrangeas that spreads wherever hydrangeas are grown in Japan, and determined that the disease is caused by the JHP-phytoplasma. In our previous study, we observed that hydrangea flowers infected by the JHP-phytoplasma become green but with variability (Kesumawati et al., 2006). We clarified that this variability in flower color is caused by the difference in the JHP-phytoplasma concentration in the plant.

Phytoplasmas are the smallest self-replicating life forms on earth and are characterized by the lack of a firm cell wall and by an extremely small size (Razin et al., 1998); their concentration in infected plants is very low (Melamed et al., 2003). Phytoplasma concentration changes depending on seasonal environmental conditions (Garcia-Chappa et al., 2003) and temperature (Kaminska et al., 2000).

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The presence of phytoplasmas during the phyllody (the development of floral parts into leafy structures) and virescence (the development of green flowers and the loss of normal flower pigments) of hydrangeas has been reported in Europe (Muller, 1971; Welvaert et al., 1975), USA (Hearon et al., 1976), France (Cousin et al., 1986) and Japan (Kanehira et al., 1996; Sawayanagi et al., 1999).

In Japan, green hydrangea flowers have a potential market as cut flowers, dried flowers or potted plants and are sold at a high price. An observation in the field showed that the inflorescences of hydrangeas grown under the shade of neighboring plants are always green. This is important information for inducing flower greening. To date, no studies of the relationship between shading and flower color in phytoplasma-infected hydrangeas have been conducted. In this study, the effect of light intensity (varied by shading with cheesecloth) on flower greening in hydrangea plants infected by the JHP-phytoplasma was examined.

2. Materials and Methods

2.1. Plant materials and shade conditions

2.1.1. First-year experiment

The experiment was conducted at Kyoto University experimental farm, Japan (latitude, 35°01′N; longitude, 135°47′E). Two 'Midori' plants infected by the JHP-phytoplasma were used as stock plants for propagation. Thirty 10-cm-long shoot cuttings from each of a single stock 'Midori' plant that was infected by the JHP-phytoplasma were propagated in August 2003. Twenty-four cutting from each of three hydrangea cultivars 'Libelle', 'Rosea' and 'Madame E. Mouillere' were individually grafted to a rootstock from another single 'Midori' plant that was also infected by the JHP-phytoplasma. All the shoots and grafted cuttings were planted in trays containing vermiculite and placed under mist condition.

In September 2003, twenty-five 'Midori' plants and five plants each of 'Libelle', 'Rosea' and 'Madame E. Mouillere' in the same growth stage were transferred and grown in 15-cm-diameter pots filled with potting soil (Metro Mix 360 Scotts Co., Marysville, Ohio). Transplants were grown in a greenhouse under natural photoperiod conditions at 35/17 °C (max/min). Each plant was fertilized weekly with 1 L of a solution containing 1.25 g L^{-1} water-soluble 5N–10P–5K fertilizer (Hanakoujou, Takeda, Tokyo, Japan). All the plants were irrigated with tap water on nonfertilization days.

In March 2004, the plants were divided and grown in the field and covered with black cheesecloth. Shading percentage was expressed as an increase in the number of sheets of cheesecloth used in shading the plants from sunlight. The shade conditions in this experiment were full sunlight intensity (100% sunlight intensity), shading with one sheet of cheesecloth (70% sunlight intensity) and shading with two sheets of cheesecloth (49% sunlight intensity). Light intensity was measured by Luxmeter (UNITEST Digital Luxmeter 93408). There was no significant difference in temperature among the shading conditions.

2.1.2. Second-year experiment

In March 2005, we repeated the treatment under full sunlight intensity and shading with two sheets of cheesecloth. 'Midori' plants that were infected by JHP-phytoplasma from first year experiment were used in the second-year experiment. Six potted plants each of the JHP-phytoplasma-infected 'Midori' plants were grown under full sunlight intensity and shading with two sheets of cheesecloth.

2.2. Preparation of the JHP phytoplasma non-infected plants

The infected 'Midori' plants were grown in a greenhouse. In April 2002, to produce JHP-phytoplasma-free 'Midori' plants, vegetative shoots were collected from these plants and sterilized for 15 min in 0.05% sodium hypochlorite and washed three times with sterile water. Uniform-size shoot tips with two leaf primordia were dissected. The excised shoot tips were cultured on a modified Knop medium containing Knop's macroelements at half strength (Knop, 1865) plus ferric-ethylenediaminetetraacetate (Fe-EDTA) (Murashige and Skoog, 1962), Ringe and Nitsch's microelements plus organic acids, and 20 g L^{-1} sucrose. This medium was solidified by adding 3 g L^{-1} gellan gum. Prior to autoclaving at 121 °C for 15 min, 0.5 ppm of plant preservative mixture (PPM) (Nacalai, Kyoto) was added to the medium to prevent bacterial proliferation; the medium's pH was adjusted to 5.8. After autoclaving, 5 mL of modified Knop medium was solidified in a 6-cm-diameter petri dish. Shoot tips were cultured at 20 ± 3 °C under a 16-h photoperiod with a light intensity of 70 μE m^{-2} s^{-1} provided by cool white fluorescent tube lights. After buds arose, they were subcultured on modified Murashige–Skoog (MS) medium containing MS macroelements plus Fe-EDTA (Murashige and Skoog, 1962) and Ringe and Nitsch's microelements plus organic acid (Ringe and Nitsch, 1968) for shoot elongation.

In March 2004, two-year-old plants from the JHP-phytoplasma-free 'Midori' buds that arose from the shoot tips were transplanted to 15-cm-diameter pots filled with potting soil (Metro Mix 360) and grown in a greenhouse under the same conditions as those for the first experiment.

The JHP-phytoplasma detection test was performed on the buds that arose from the shoot tips using PCR analysis.

On the other hand, twelve shoot cutting from each of three hydrangea cultivars 'Libelle', 'Rosea' and 'Madame E. Mouillere' were taken from stock plants that were free from JHP-phytoplama. All of the shoots were planted in trays containing vermiculite, placed under mist conditions and used for comparison with grafted plants that were infected by JHP-phytoplasma.

2.3. Measurement of green-area ratio of individual flowers in inflorescence

The green-area ratio of individual flowers in an inflorescence was measured according to Kesumawati et al. (2006). A color detector with fluorescent light (Taketombo Co., Ltd., Japan) was used for taking a photograph of each flower separated from an inflorescence in a dark room. The photographs of individual flowers were taken using a digital camera at an Focus (F) of 5,6 and a shutter speed (S) of 1/25.

Green-area ratio of each flower from an inflorescence was measured from a photograph using Adobe Photoshop with the fuzziness at 135. The green-area ratio of each flower was measured by determining the number of green pixels divided by the total number of pixels of the total flower area. From the green-area ratio of the flowers, we classified the flowers into four types: green-area ratio = 0, completely blue/pink/white flowers; $0 <$ green-area ratio < 0.4, blue/pink/white-green flowers; $0.4 \leqq$ green-area ratio < 0.8, greenish flower; $0.8 \leqq$ green-area ratio, completely green flower (Fig. 1).

2.4. Data collection and calculation

Flowers were collected from inflorescences of all 'Midori' plants and grafted plants under different conditions. In the first-year experiment, six potted 'Midori' plants were used for data collection and in this experiment used randomized block design. In the second-year experiment, all individual plants were used again for data collection.

The data for the grafted plants 'Libelle', 'Rosea' and 'Madame E. Mouillere' were collected from one potted plant for each under