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Petal blackening and lack of bud opening in cut lotus flowers (*Nelumbo nucifera*): Role of adverse water relations

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ABSTRACT

Lotus flowers (*Nelumbo nucifera* Gaertn.) are commercially sold as closed buds. When placed in water the buds fail to open and the outer petals show rapid blackening. We investigated whether this is due to adverse water relations. Placing a plastic bag over the flower head delayed petal blackening, indicating that it was induced by early water stress. This treatment did not result in bud opening. A rapid occlusion of the stem xylem was found. Four possible causes of this occlusion were investigated:air uptake into the xylem, microorganisms in the vase solution, a plant-induced effect, and exuded latex. Preventing the uptake of air into the stem ends did not affect water uptake. Inclusion in the vase water of antibacterial compounds, or antioxidants that inhibit the plant-induced xylem blockage in other species, similarly did not alleviate the xylem occlusion. Cut stems exuded copious latex, close to the opened xylem conduits. Latex exudation was prevented by cutting under water, allow the latex to flow out, and cut again in air, within 1 cm from the previous cut. This treatment did not promote water uptake of the cut stems. A pulse treatment with citric acid also reduced latex flow, but also did not prevent the decrease in water uptake. Treatment with ethephon or GA_3 delayed the xylem occlusion, which suggests that it is induced by the plant itself. Only GA_3delayed petal blackening. None of these treatments promoted flower opening. It is concluded that adverse water relations are a cause of early petal blackening in cut lotus, but is not a cause of the lack of bud opening. The adverse water relations are apparently due to a plant-induced xylem occlusion which is different from those studied thus far in other species.

1. Introduction

Lotus plants (*Nelumbo nucifera* spp. nucifera) are mainly found in Asia. The leaves and the flower stalk emerge from a tuberous rhizome that grows in the sediment of a body of water, usually 0.5–1.0 m deep. The flowers are single and develop at the apex of an erect floral stalk (peduncle). The peduncle has become lignified by the time of bud opening (Mosely and Uhl, 1985; Hayes et al., 2000). In Thailand one of the two main commercial cultivars has several green outer petals and many white inner petals. It is locally called cv. Saddabutra, and is probably identical to cv. Album Plenum.

If left uncut, the full-grown floral bud opens in the morning and closes at night, for a few consecutive days. In uncut flowers the time between bud opening and the end of floral life-span is usually about 4–5 days. In intact plants the end of floral life is determined by petal abscission. Some signs of petal blackening can sometimes be observed by the time of petal abscission.

Floral buds are harvested just before they would open (mature bud stage) and are transported dry and subsequently placed in the temple by the religious devotee. When the cut stems are placed in water the buds do not open and the outer petals show early blackening. We investigated whether opening could be promoted and whether the petal blackening symptoms could be delayed. Imsabai et al. (2010) reported that treatment with 1-methylcyclopropene (1-MCP), an inhibitor of the ethylene receptor, reduced the rate of bud ethylene production and delayed the onset of petal blackening by about 2 days. 1-MCP did not promote the opening of the floral bud. These data suggested that ethylene is a cause of petal blackening but not of the lack of bud opening. Additionally, Imsabai and van Doorn (2013) reported that cytokinins and gibberellic acid (GA_3) delayed petal blackening but had no effect on bud opening.

Here, we tested the hypotheses that the lack of bud opening and the early petal blackening in lotus flowers are due to adverse water relations. Many species of cut flowers, even with their stems placed in vase water, rapidly exhibit serious water stress. This is due to a xylem occlusion in the stem. Depending on the species, the xylem occlusion can be due to factors such as bacteria in the vase water, a plant-induced effect, and the presence of air bubbles in the xylem

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conduits (van Doorn, 1997, 2012). Furthermore, the stems of lotus flowers exude copious latex after cutting. The latex flows into the vase solutions and might be taken up with the vase water and then block the xylem. We tested if lotus flowers showed such a xylem occlusion, and if so, whether it caused early petal blackening and lack of flower opening. It was observed that petal blackening was delayed by decreasing water stress and that a hitherto not described type of xylem occlusion induced this water stress.

2. Materials and methods

2.1. Plants

Lotus flower buds (*Nelumbo nucifera* Gaertn., cv. Saddhabutra, likely the same as cv. Album Plenum) were harvested in the morning. The buds were picked at their normal commercial stage, i.e. with the floral buds still fully closed but about to open. Workers walked in the water of the lotus pond, which stood about 1 m deep, or collected stems by boat. Stems were broken under water, close to their junction with the rhizome. Directly after harvest, the stems were held dry or were placed in purified water (tap water, after passing through reverse osmosis equipment). Stem length at harvest varied from 40 to 60 cm. Stems were brought to the laboratory within about 1 h of harvest. In the laboratory the stems were recut in air, to a length of 25 cm, and then placed individually in glass vials or glass graduated cylinders, containing purified water. The flower stalks were held in a temperature-controlled room at 25 °C, 65–75% RH, and natural light supplemented with TL light (light from about 7 a.m. to 7 p.m., with total photon flux density about 15 μmol m^{-2} s^{-1}). In some tests, the flower stalks with buds were brought to the laboratory as described, and were then held dry at 20 °C for 24 h, after which the stems were recut in air to a length of 25 cm, removing about 30 cm. This was compared with the same treatment without the period of dry storage.

2.2. Petal blackening and flower opening

During vase life, petal blackening was assessed visually, at the end of the morning. The length of vase life was defined as the period until half of the visible petals showed black patches. Flower opening was determined visually. The flower was defined to open if the petals left an opening at their tips.

2.3. Chemicals

Treatment with citric acid has been described as a means to stop latex flow from cut stems (van Doorn, 1997). Citric acid (Sigma–Aldrich) at 150 mg L^{-1} was used as a pulse treatment, just before vase life, or was continuously included in the vase water during vase life.

The following antibacterial chemicals were included in the vase water at the onset of vase life and were not renewed: hydroxyquinoline sulphate (HQS; from Sigma–Aldrich, at 100, 200, 300, or 400 mg L^{-1}), sodium dichloroisocyanuric acid (DICA; from BDH, at 10, 20, 30, 40, or 50 mg L^{-1}), or silver nitrate (Sigma–Aldrich, at 25, 50, 75, or 100 mg L^{-1}).

In other tests, some chemicals were used that are known to prevent cutting-induced xylem occlusion in the stems of cut flowers of other species (van Doorn and Cruz, 2000; Vaslier and van Doorn, 2003; Loubaud and van Doorn, 2004). Several of these chemicals were included in the vase water at the onset of vase life, thus were tested as a continuous treatment. These chemicals were *n*-propylgallate (1, 2, and 5 mM), phloroglucinol (0.1 and 0.5 mM), *n*-nitrophenol (1 mM), *p*-nitrophenol (4-nitrophenol, 1, 2, and 5 mM), and phenylhydrazine (0.1 and 0.5 mM). Tropolone was tested continuously at 0.25 and 0.50 mM. 4-Hexylresorcinol, included in purified water at 2 and 10 mM, was given as a 5 h pulse treatment, prior to placement of the stems in purified water. In other tests it was applied continuously at 4, 40 and 400 μM. These chemicals were obtained from the same sources as mentioned by van Doorn and Vaslier (2002). *S*-carvone, the main etherical oil from caraway seed, was tested continuously at 32 and 64 μM. It was obtained from Sigma–Aldrich.

Gibberellic acid (GA_3; PhytoTechnology Laboratories, Shawnee Mission, KS, USA) was applied at 0.12 mM. The cytokinins benzyladenine (BA; Sigma–Aldrich) and thidiazuron (TDZ; PhytoTechnology Laboratories, Shawnee Mission, KS, USA) were tested at 0.1 mM and at 2.5 μM, respectively. Ethephon (ProCrop Co. Ltd., Thailand), which releases ethylene, was applied at 200 mg L^{-1}. This treatment reduced vase solution pH to 2.9. Controls for pH 2.9 were prepared using 1 M HCl, or HEPES buffer (Sigma–Aldrich), whereby the pH was adjusted with 1 M HCl. HEPES buffers were also applied at pH 3, 4, 5, 6, and 7. These chemicals were included in the vase solution at the onset of vase life and were not replenished. Finally, the surfactants Tween-20 and Agral-LN were tested at 0.01, 0.1 and 1.0 mL L^{-1}.

2.4. Water uptake, transpiration, water balance, and fresh weight

The rate of water uptake was measured by placement of stems in graduated cylinders and daily measuring of the solution level. The rate of transpiration was assessed by weighing. The water balance (the difference between the rate of water uptake and transpiration) was calculated from the uptake and transpiration data. Flower fresh weight (FW) was determined by weighing. Results were expressed as the percentage of initial FW.

2.5. Covering with plastic bags

Polypropylene (PP) bags, 12.7 cm × 15.2 cm, were used to cover the flower heads. The open end of the bag was wrapped around the stem but was not tied to the stem. Four or eight small holes (diameter about 0.55 mm) were made by using a needle.

2.6. Stem anatomy and latex flow

Stems were cut and the exudation of latex was observed, using a binocular microscope magnifying 10 times. Stem anatomy was studied by cutting thin slices by hand and observation under a microscope. Latex flow was determined by removal of the exuded latex and weighing.

2.7. Preventing latex flow and air uptake

When stems were cut at 1 cm or more from the original cut surface, new latex was observed to flow out. However, when re-cutting the stems in air at 0.5 cm or less from the initial cut, no new latex flowed out. This difference was used to test whether exudation of latex is a cause of early petal blackening. The stems were again cut in air, removing 0.5 cm (no new latex flow), and stems were immediate placed in water. This was compared with recutting the stems at 2 cm from the original cut surface (resulting in latex flow into the water) and immediate placement in water. Another method to reduce latex flow involved placement of freshly cut stems in an aqueous citric acid solution at 150 mg L^{-1} (pH about 3.3).

We prevented any air from entering the stem end by recutting the harvested stems under water, gently shaking the ends for 3 min to allow the exuded latex to flow off. The stem end was then placed in a cup, under water. Stems were brought to a bucket of water while inside this cup. The stems were transported to the laboratory in this bucket with water. In the laboratory a graduated cylinder was held under water in the bucket and the stem was placed (under