Agriculture and Natural Resources 50 (2016) 32–37

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original article

# Role of carbohydrates in petal blackening and lack of flower opening in cut lotus (*Nelumbo nucifera*) flowers

Petcharat Netlak,[a, b] Wachiraya Imsabai[a, b, c, *]

[a] Department of Horticulture, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand
[b] Center for Advanced Studies for Agriculture and Food, KU Institute for Advanced Studies, Kasetsart University (CASAF, NRU-KU), Bangkok 10900, Thailand
[c] Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok 10400, Thailand

ARTICLE INFO

*Article history:*
Received 9 October 2014
Accepted 8 June 2015
Available online 11 February 2016

*Keywords:*
Fructose
Glucose
Petal abscission
Sucrose

ABSTRACT

Lotus flowers (*Nelumbo nucifera* Gaertn.) are sold as stems with terminal buds that are about to open, to be used in Buddhist religious offerings. The buds fail to open if the cut stems are placed in water. Moreover, the petals rapidly turn black. This study investigated whether this might be due to a lack of carbohydrates. The inclusion of different sugars in the vase water, together with an adequate antimicrobial compound, had no effect on petal blackening and did not promote flower opening. By contrast, cutting the buds at a slightly more mature stage of development resulted in full flower opening. However, the levels of glucose, fructose, or sucrose in the white petals were the same at the later date of harvest as at the earlier date; thus, this did not explain the effect of cutting at an advanced stage of development. It was concluded that a lack of sugars does not seem to explain petal blackening or a lack of flower opening in lotus flowers that are cut at the normal harvest stage (bud stage).



## Introduction

Lotus flowers (*Nelumbo nucifera* Gaertn.) are commercially sold as closed buds. The stems are brought to a Buddhist temple as an offering. If the stems are placed in water, the buds do not open, and the outer petals rapidly turn black. Treatment with 1-methylcyclopropene (1-MCP), an ethylene antagonist, delayed petal blackening and this indicated that blackening is regulated by endogenous ethylene (Imsabai et al., 2010). Blackening was delayed after treatment with hormones such as gibberellic acid and cytokinins (Imsabai and van Doorn, 2013), and was delayed by decreasing the rate of transpiration (Imsabai et al., 2013). These data suggested that the early blackening was partially due to adverse water relations, and by a low level of hormones such as gibberellic acid and cytokinins. However, none of these treatments caused bud opening.

After harvest, the cut lotus stalk with its terminal floral bud has no leaves, thus is not in contact with a potential source of carbohydrates. Since the stems are placed at relatively low light levels (about 15 μmol/m$^2$/s), net photosynthesis is likely absent. This means that petal sugar levels might decrease. This might be a cause of early blackening and the lack of flower opening. In previous studies using a large range of cut flowers, it was found that an exogenous carbohydrate supplementation is adequate to delay senescence. Many floral preservative solutions containing sucrose and a germicide as these are considered essential to extend the vase life of cut flowers (Halevy and Mayak, 1981). These preservatives also promoted flower bud opening, the lack of which occurs in several cut flowers such as roses (Goszczynska et al., 1990; Kuiper et al., 1995; Figueroa et al., 2005), tuberose (Hutchinson et al., 2003), and gladiolus (Marousky, 1968; Mayak et al., 1973).

The current study tested the hypothesis that low sugar levels in the petals account for the lack of bud opening and the early petal blackening in bud-cut lotus flowers.

## Materials and methods

### *Plant materials*

Flower buds of lotus (*N. nucifera* Gaertn., cv. Saddabutra, most likely identical to cv. Album Plenum) were harvested in the morning. The buds were picked at their normal commercial stage, that is, with the floral buds still fully closed but expected to open in

* Corresponding author. Department of Horticulture, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand.
*E-mail address:* agrwyi@ku.ac.th (W. Imsabai).

http://dx.doi.org/10.1016/j.anres.2015.06.001

about a day, if they had not been picked. Workers walked in the water of the lotus pond, which stood about half a metre deep, or collected stems by boat. Stems were broken under water, close to their junction with the rhizome. After harvest, the stems were held dry for at most 15 min or were immediately placed in purified water (tap water, after passing through a reverse osmosis apparatus). The stem length at harvest varied from 50 to 70 cm. Other flowers were harvested at slightly later stages, when the flower were about to open or had already opened, and the tip of the petals showed a gap of about 0.5–1.0 cm. Stems placed in purified water were brought to the laboratory within about 1 h of harvest. In the laboratory, the stems were recut in the air, to a length of 30 cm, then were placed individually in glass jars with purified water. The stems were held in a temperature-controlled room at 25 °C, about 70% relative humidity, and, unless otherwise indicated, natural light was supplemented with light from tungsten lamps (light from about 07.00 am to 07.00 pm; photon flux density 15 μmol/m$^2$/s).

*Bud opening and petal blackening*

The opening of floral buds was determined by measuring the maximum diameter of the gap at the tip of the petals, using a ruler. Abscission of petals was determined by measuring the time until half of all petals of a flower had fallen. Petal blackening was assessed by the time until half of the petals on a flower showed black patches. Observations were made daily at 09.00–10.00 am.

*Levels of free carbohydrates*

The soluble sugar content was determined using the method of Reyes et al. (1982), with slight modification. One gram fresh weight of lotus petal was ground with a cooled mortar and pestle. Five ml of 80% ethanol were added after which the mixture was incubated in a water bath at 80°C for 30 min. The extraction mixture was filtered through four layers of cotton cloth, then through a nylon membrane filter (0.45 μm pore size). The filtrate was freeze-dried under vacuum, re-dissolved in 5 mL of double deionized water and filtered through a nylon membrane filter (0.22 μm pore size). The soluble sugar content were determined and quantified by high performance liquid chromatography using a C18 column (Sugar Pak I; Waters; Milford, MA, USA) and a refractive index detector (RID-10A; Shimadzu; Kyoto, Japan). The filtered extract and 50 mg/L of calcium ethylenediamine tetraacetate (CaEDTA; Merck; Darmstadt, Germany) was pumped through the column at a flow rate of 0.5 mL/min and the temperature was adjusted to 90°C. Pure standards of sucrose (Merck; Darmstadt, Germany), D-glucose (BDH; Leuven, Belgium), and D-fructose (BDH; Leuven, Belgium) were identified by their retention times and were quantified by applying a range of known concentrations.

Sugar levels were determined in the outer, greenish petals, of stems cut at stage 5 (Fig. 1) and in the inner, white petals, in stems cut at later stages of development. In intact plants, sugar levels were determined at various stages of development, both in the outer and in the inner petals.

*Inclusion of chemicals in the vase water*

Sucrose, glucose, fructose, and 8-hydroxyquinoline sulphate (8-HQS) were obtained from Merck (Leuven, Belgium), BDH (Leuven, Belgium), BDH and Sigma–Aldrich (St Louis, MO, USA), respectively. Chemicals were included in the vase water at the onset of vase life and were not replenished. Glucose, fructose, or sucrose were applied at 5, 10, 15, 20, 25, 50 and 100 g/L, together with 200 mg/L 8-HQS as an antimicrobial compound. The 8-HQS was also tested separately.

*Statistical analysis*

All experiments in which flower opening, petal blackening, or petal abscission was measured used 8–10 flowers per treatment in a completely randomized design. Data of sugar treatments were tested using analysis of variance and an *F*-test at $p \leq 0.05$. Biochemical analyses used three biological replications, and mean values of treatments were analysed by the least significantly difference. Most of the experiments were repeated at a later date.

## Results

*Developmental stages of floral buds; effect of sugar feeding*

The developmental stages of the floral buds and open flowers, as used in the present experiments, refer to flowers that remained attached to the plant. The identified stages are shown in Fig. 1 and are based on diameter classes.

Exogenously applied sugars might alleviate the effects of a lack of endogenous sugars, whereby some sugars might be taken up or used more efficiently than others. Glucose, fructose or sucrose were included in the vase solution at a range of concentrations in order to be able to find the optimum one. Sugars were applied together with 200 mg/L 8-HQS as an antimicrobial compound. The 8-HQS at 200 mg/L had no effect on the time to petal blackening or on flower opening (Fig. 2). The sugar treatments did not delay petal blackening, except in a few tests in which blackening in the controls was found extremely early (already by day 3). When applied at 50 g/L, each of the sugars had no effect, but rather hastened blackening, depending on the experiment. Petal blackening was invariably hastened when sugars were applied at 100 g/L (Fig. 2).

The sugar treatments had no effect on bud opening, either in flowers harvested when the buds were still tightly closed or if


**Fig. 1.** Stages of bud and flower development of the sacred lotus (*Nelumbo nucifera* spp. nucifera) cv. Saddabutra, as defined in the present paper. The stages were based on bud and flower diameter in attached buds and flowers. Buds at stage 1 had a diameter 2.0–2.5 cm, stage 2 a diameter of 3.0–3.5 cm, stage 3 a diameter of 4.0–4.5 cm, etc. The buds that were about to open were called stage 5 (diameter 6.0–6.5 cm), which is the commercial cutting stage. Flowers that had just opened (having an opening at the petal tips) were called stage 6 (diameter 7.0–7.5 cm). Flowers that had an opening of about 3–4 cm at their tips were at stage 7 (diameter 8.0–8.5 cm). The stage just before the abscission of the last (green) outer petals was called 8 (diameter 9.0–9.5 cm). The last of the (white) inner petals were about to abscise by stage 10 (diameter 11.0–11.5 cm).