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Research note

Opening of cut *Iris x hollandica* flowers as affected by temperature, dry storage, and light

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ABSTRACT

Flower opening in *Iris* (*Iris x hollandica*) depends on elongation of the pedicel + ovary. This elongation lifts the bud above the point where the sheath leaves no longer mechanically inhibit lateral tepal movement. We here report on the effects on flower opening of storage at various temperatures, of holding the flowers dry rather than in water, and of a 12 h light/dark cycle instead of darkness, in cv. Blue Magic. During 3 d of storage in darkness at 11 °C or 6 °C the flowers placed in water opened. Flowers stored at 3.0 °C did not open during the storage period but did so during subsequent vase life at 20 °C. Flowers stored in water at 0.5 °C remained closed, even during subsequent vase life at 20 °C. None of the flowers that were stored dry for 3 d at 15 °C, 11 °C, 6 °C, 3 °C or 0.5 °C opened during vase life. Compared to flowers placed in continuous darkness, a rhythm of 12 h light and 12 h darkness inhibited opening during a 3 d storage period at 20 °C. It is concluded that cut *Iris* flowers (a) can be stored in water at 3 °C for more than a week, but cannot be stored for 3 d or more in water at 15 °C, 11 °C, 6 °C or 0.5 °C, and (b) cannot be stored dry for long (under the present conditions 3 d or longer) at any of these temperatures. *Iris* flowers were found to be chilling-sensitive, although only at temperatures of about 0.5 °C.

1. Introduction

Flower opening usually requires elongation growth to increase petal (or tepal) size and movements. Opening is affected by various factors, including temperature, drought stress, light quantity and quality (Reid and Evans, 1986; van Doorn and van Meeteren, 2003).

The opening of *Iris* floral buds is different from that in most other flowers (Mayak and Halevy, 1971; Reid and Evans, 1986; Macnish et al., 2010). By the time the tepal tips of the buds are visible the flower is ready to open. However, opening is initially impeded as the bud is tightly enclosed by the uppermost green leaves, which mechanically impede the opening movements. Elongation growth in the subtending ovary and pedicel is therefore required for bud opening in *Iris*. This elongation lifts the base of the floral bud to a position where the two upper leaves no longer block opening. *Iris* flower opening was highly correlated with the growth of the pedicel and ovary (Çelikel and van Doorn, 2012; van Doorn et al., 2013).

Iris flowers are often brought in water to the flower auction, but can be held dry during subsequent stages of the distribution to the consumers. It is believed, however, that dry storage is detrimental for flower opening. At the premises of the growers the flowers are stored in water at about 4 °C, but it is not obvious that this is the optimal temperature. The flowers are also stored in darkness at the premises of the growers, but it is not known if darkness is to be preferred over storage in the light. Darkness is known to promote elongation growth in plants, thus might lead to precocious flower opening in *Iris*. In preliminary experiments it was observed that flowers that had been stored in water in darkness at 11 °C or 6 °C opened earlier during vase life than flowers that had not been stored, i.e. kept in water in a 12 h dark/12 h light cycle at 20 °C. This suggested that continuous darkness promoted flower opening.

We previously reported that dry storage of *Iris* flowers, for 1 or 2 d at 20 °C, inhibited elongation of the pedicel and ovary, and associated flower opening (Çelikel and van Doorn, 2012). Here we investigated the effects on flower opening of temperature during storage in water, and during dry storage. We also tested the effect of storage in the light, compared to darkness.

2. Materials and methods

2.1. Plants

Iris flowers (*Iris x hollandica* Tub., cv. Blue Magic) were harvested at commercial growers in the Netherlands. They were picked at about 06:00, at a stage whereby the tepals tips were just visible (day

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0 of flower opening; commercial cutting stage), or, in only a few tests, when the tepal tips were just not yet visible (immature buds). Immediately after harvest the flowers were placed in water and stored in a 4 °C room (about 3 h) until transport to the laboratory, the same morning. During transport by car (non-refrigerated) the stem ends were stood in water. Transport took less than 2 h. In the laboratory the stems were recut in air, to a length of 45 cm.

After the storage treatments (discussed below), flowers were stood individually in glass vials in a climate-controlled room at 20 °C, 60% RH, and a photosynthetically active photon flux of 15 μmol m^{-2} s^{-1} (Philips TDL 36 W/84 cool white fluorescent tubes) from 7 a.m. to 7 p.m.

2.2. Effects of storage at various temperatures, comparing storage with stem ends in water or without water

One storage experiment was carried out at 11 °C, 6 °C and 0.5 °C, with sampling on days 3, 6 and 9. In the room at 0.5 °C the temperature oscillation had a low amplitude and did not reach 0 °C. In preliminary experiments it was observed that placing flowers horizontal inhibited flower opening. During storage the stems were therefore placed vertically in glass vials containing either deionised water or no water. Other storage experiments were at 20 °C, 15 °C, 3 °C or 1 °C. After storage the stems were placed in deionised water at 20 °C in the climate-controlled room.

To determine water loss the flowers were also placed upright in glass vials without water. The flowers were held dry, at the conditions described above, for one or two days. After the period of dehydration the stems were recut in air to a length of 40 cm, unless otherwise indicated, and were placed in deionised water in the climate-controlled room.

2.3. Effects of a 12 h light/dark cycle

Most storage experiments were in darkness. To test the effect of light two perspex chambers (0.7 m × 0.7 m × 0.7 m) were placed in the climate-controlled room. One of these boxes was entirely covered with black plastic, thus was dark inside, the other was left uncovered, thus was exposed to the 12 light/dark cycle of the room. The temperature in the chambers was 20 °C and the RH was about 99%. Flowers were placed in the closed chambers, vertically, with their stems in graduated cylinders with water. A set of controls was placed outside the perspex chambers, thus at 20 °C and 60% RH. After the treatments the flowers were placed in the climate-controlled room. About 1 cm of the tips of the tepals of the closed buds was visible at the onset of the experiment (mature buds). In another series the tepal tips were just not yet visible (immature buds). Other tests compared continuous darkness with continuous light, at 20 °C, or did so at 3 °C.

The following experiments were carried out. Exp. 1, Flowers were stored in darkness, in water or held dry, for 3, 6 or 9 d, at 11 °C, 6 °C or 0.5 °C. Exp. 2, Flowers were stored in darkness, in water or dry at 3 °C or 1 °C, for 3 or 11 d. Exp. 3, Flowers were stored dry in darkness at 15 °C and 20 °C for 1, 2 and 3 days. Exp. 4, Flowers were stored in water at 20 °C for 3 d, either in darkness, or in 12 h darkness and 12 h light. Exp. 5, Flowers were stored in water at 20 °C for 3 d, either in darkness, or in continuous light. In Exp. 6 the same treatments as in Exp. 5 were applied at 3 °C.

2.4. Evaluation of pedicel elongation, flower opening, and fresh weight

The increase in pedicel + ovary length during storage was determined by measuring the distance between the uppermost stem internode and the tepal tips of the closed buds. The maximum length of the pedicel + ovary, during vase life, was measured using a calibrated ruler.

The tepals in floral buds are still vertical and pressed to each other. Their opening angle is therefore zero. Opening of the flower is due to lateral movement of the tepals, which occurs at the tepal base. During opening the tepal bases thus show an increasing angle with regard to each other. We determined this angle, using a calibrated protractor. The angle was determined between two randomly chosen tepals. A second measurement used a different set of tepals. The two measurements, which often differed by about 5–10°, were averaged. When the buds are still closed the protracted ruler showed an opening angle of 10°, owing to the thickness of the petal bases.

Fresh weight (FW) was determined during the course of storage, by weighing 10 individual flowers at daily intervals.

2.5. Statistics

All treatments included ten replicate flowers. Results were compared by analysis of variance using the GENSTAT V program (Rothamsted, U.K.) and F test at $P \leq 0.05$. The experiments were repeated at least once. The data shown are from one of the repeat experiments.

3. Results and discussion

3.1. Effects of temperature and dry storage in darkness

In Exp. 1 (see Section 2.3) flower FW increased in flowers stored in water, more so at higher temperature of storage. On day 3 of storage the FW increase was 5.2, 2.4 and 0.5% at 11 °C, 6 °C or 0.5 °C, respectively. At 9 d of storage these percentages were 6.3, 5.0 and 0.5%, respectively. Flower FW decreased in flowers that were stored dry, almost independent of storage temperature. After 3 d of storage FW had decreased by about 11% and by 9 d by about 18% (data not shown).

During the first 3 d of storage in water, the growth of the pedicel + ovary was positively correlated with the storage temperature (Fig. 1A). In dry stored flowers, the growth of the pedicel + ovary was considerably lower than in flowers stored in water, but was also correlated with the storage temperature (Fig. 1B).

The final length of the pedicel + ovary was measured on day 6, i.e. after flower senescence. This final length was higher after storage in water at higher temperature (Fig. 1C). The final length of pedicel and ovary was negatively affected by dry storage, more so upon a longer period of storage. This inhibitory effect was independent of storage temperature (Fig. 1D). Flowers opened well after each of the storage periods in water at 11 °C or 6 °C, but did not open after any period of storage at 0.5 °C (Fig. 2A). Flowers also did not open after dry storage, at any of the temperatures tested (Fig. 2B).

In Exp. 2 (see Section 2.3), the pedicel + ovary had grown by 1.4 cm after 3 d of storage in water at 3 °C. After storage in water for 11 d these parts had grown by 3 cm, compared with day 0 (data not shown). Compared to flowers that had not been stored, storage of flowers in water at 3 °C (in darkness) for 3 or 11 d did not affect the maximum length of the pedicel + ovary and did not influence maximum flower opening, during vase (data not shown). Dry storage at 3 °C or 1 °C, for 3 or 11 d, resulted in inhibited elongation of the pedicel + ovary and in less flower opening, during subsequent vase life at 20 °C (data not shown).

In Exp. 3, differences between one, two and three days of dry storage were investigated at 15 °C and 20 °C. During vase life the flowers opened after 1 day of dry storage at these temperatures but the opening was less than in the controls in water (Table 1). After two days of dry storage at 15 °C only 5 out of 10 flowers opened

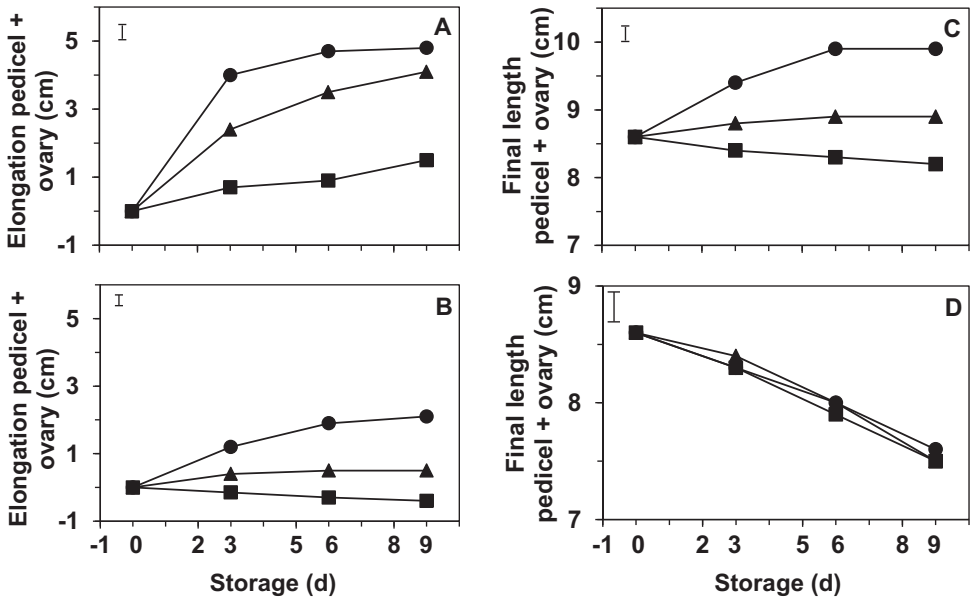

Fig. 1. Elongation growth of the pedicel + ovary during storage (A and B) and the final length of the pedicel + ovary during subsequent vase life (C and D) of cut *Iris x hollandica* flowers. Flowers with a stem length of 45 cm, with all leaves attached, were stored for 0, 3, 6 or 9 d in water (A) or were stored dry for these periods, placed vertical (B). After these periods of storage the flowers were placed in vases at 20 °C to determine opening and maximum length of the pedicel + ovary. Storage temperatures were 11 °C (●), 6 °C (▲) and 0.5 °C (■). Data are means of 10 replicate flowers. $LSD_{0.05}$ is indicated in the upper left corner of the graphs.

during vase life, while after this period of dry storage at 20 °C only 2 out of 10 opened. No flower opening was found during vase life after dry storage for 3 d (data not shown). The effects on opening were well correlated with those on pedicel + ovary length (data not shown).

The present experiments confirmed the previous finding that flower opening in *Iris x hollandica* was, in general, highly correlated with elongation of the pedicel and ovary. This elongation was required for flower opening (Reid and Evans, 1986; Çelikel and van Doorn, 2012). The present data also show that elongation

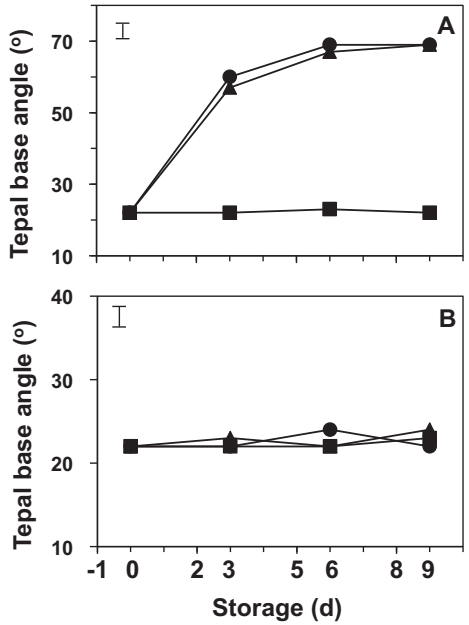

Fig. 2. Maximum flower opening during vase life at 20 °C in cut *Iris x hollandica*, after storage. Opening is expressed as the angle between tepal bases. Flowers with a stem length of 45 cm, with all leaves attached, were stored for 0, 3, 6 or 9 d in water (A) or were stored dry for these periods, placed vertical (B). Storage temperatures were 11 °C (●), 6 °C (▲) and 0.5 °C (■). Data are means of 10 replicate flowers. $LSD_{0.05}$ is indicated in the upper left corner of the graphs.

Table 1
Effects of 3 days of continuous darkness and 3 days of a 12 h dark/light cycle, on flower opening in cut *Iris x hollandica* cv. Blue Magic flowers, with a stem length of 45 cm and all leaves attached. At the onset of the experiment flowers either showed the tips of the tepals of the closed bud (mature buds) or were about to show the tips (immature buds). Flowers were in a climate-controlled room at 20 °C and 15 μmol m^{-2} s^{-1} from Philips TDL 36 W/84 cool white fluorescent tubes, from 7 a.m. to 7 p.m., and either in a perspex chamber (RH about 99%) or outside the chamber (RH 60%). Two chambers were used, one in which the light of the climate-controlled room could enter from five sides, and one covered with black plastic (complete darkness). The degree of flower opening, expressed as the angle between tepal bases, and the length of pedicel + ovary were measured after 3 d of storage.

Treatments	Flower opening (d 3) (tepal base angle)[a]	Length of pedicel + ovary (d 3) (cm)[a]
Mature buds		
Darkness, 99% RH	81.2 ± 9.0 a	9.0 ± 0.2 ab
Darkness/Light, 99% RH	60.6 ± 8.5 b	8.6 ± 0.2 bc
Darkness/Light, 60% RH	63.7 ± 8.3 b	8.6 ± 0.3 bc
Immature buds		
Darkness, 99% RH	70.6 ± 8.1 ab	9.2 ± 0.2 a
Darkness/Light, 99% RH	31.9 ± 7.7 c	8.6 ± 0.2 bc
Darkness/Light, 60% RH	26.3 ± 7. 9 c	8.4 ± 0.2 c

[a] Results are means of ten replications ± SD. Data in the same column with one or more identical letters do not differ significantly ($P \leq 0.05$).

was inhibited at lower storage temperature and by dehydration during dry storage. Under specific conditions (temperature 0.5 °C in flowers stored in water, and dry storage of 3 days or more, at any of the temperatures tested) this inhibition was not reversed during vase life at 20 °C. Opening in *Iris* flowers was negatively affected at a temperature of 0.5 °C.

In most plants elongation growth is inhibited even by a rather small decrease in water potential (Kramer and Boyer, 1995). This was also found in *Iris* flowers. At an FW loss of 11% or more, pedicel + ovary elongation became severely inhibited.

3.2. Storage in darkness, under a 12 h light/dark cycle, or in continuous light

In Exp. 4 we tested the effect of a rhythm of light/darkness, during storage at 20 °C. Flowers were placed in water, for 3 d, in

continuous darkness at 20 °C and about 99% RH. This treatment was compared with flowers that were placed in water at 20 °C and approx. 99% RH that were exposed to 12 h darkness and 12 h light per day. The experiment was conducted using both flowers with normally developed buds at harvest (called mature buds), whereby the tepal tips are just visible above the green leaves, and with flowers in which the tepal tips were just not visible yet (called immature buds). Compared to the dark/light treatment, continuous darkness increased flower opening, in both mature and immature buds (Table 1). The darkness treatment resulted in higher pedicel + ovary length, measured at the end of the 3 d treatment, although only in the immature buds (Table 1). The results were compared, in the same experiments, with a treatment in which flowers were continuously placed at 20 °C and 60% RH (rather than about 99% RH) with 12 h light and 12 h dark. No effect was found of the difference between 60% RH and 99% RH (Table 1).

In Exp. 5, mature floral buds with a stem length of 45 cm were placed in water for 3 d in continuous darkness or in continuous light, at 20 °C and 60% RH. Darkness clearly promoted flower opening compared to continuous light, during the storage period. However, the effects on pedicel + ovary elongation were not statistically significant (data not shown). In Exp. 6, the same treatments as in Exp. 5 were applied at 3 °C. Opening was absent at 3 °C. No effect of light on flower opening during subsequent vase life was found (data not shown).

These data show that at storage at 20 °C in darkness promoted flower opening compared to storage in the light (either 12 per day or continuously). We found no effect of light when applied at 3 °C, on opening during vase life. The data suggest that light inhibited flower opening separate from the rate of elongation of the pedicel and ovary. Flower opening per se thus seems under the control of light. The effect is likely exerted on lateral petal opening movement. The photon flux density (photosynthetically active radiation) in these experiments most likely was below the compensation point, i.e. the light level above which net photosynthesis takes place. This means that light receptors such as phytochrome might be involved. In many species flower opening requires differential elongation growth at the tepal base (van Doorn and van Meeteren, 2003). A stimulatory effect of darkness compared to light on elongation growth has been reported in, for example, *Pisum sativa* (Behringer and Davies, 1992), *Arabidopsis thaliana* and *Brassica rapa* (Franklin and Whitelam, 2005; Pelletier et al., 2010). The effects seem mainly mediated by phytochrome, the plant pigment that signals the ratio between red and far-red light (Franklin and Whitelam, 2005; Chen and Chory, 2011).

4. Conclusions

It is concluded that cut *Iris* flowers (cv. Blue Magic) can be stored in darkness for as much as about 11 days if placed in water at about 3 °C. Storage at higher temperatures (15 °C, 11 °C, 6 °C) was not possible for as little as 3 d, and storage at 0.5 °C for 3 d resulted in lack of opening during vase life. Under practical circumstances, with flowers in bunches and wrapped in plastic or paper, the FW decrease after dry storage might be less than in the present tests. Under the present conditions, dry storage for 1 d was possible, but dry storage for 3 d, at any of these temperatures, was not. The data also show that the flowers are chilling-sensitive, although only at about 0.5 °C.

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