Water Conservation in *Kalanchoe blossfeldiana* in Relation to Carbon Dioxide Dark Fixation

Received for publication August 6, 1974 and in revised form October 31, 1974

GEORGE G. ZABKA AND S. N. CHATURVEDI

Department of Biology, United States International University, San Diego Campus, San Diego, California 92131

ABSTRACT

The succulent *Kalanchoe blossfeldiana* v. Poel var Tom Thumb was treated on long and short photoperiods for 6 weeks during which short day plants developed thicker leaves, flowered prolifically, and exhibited extensive net dark fixation of carbon dioxide. In contrast, long day plants remained vegetative and did not develop thicker leaves or exhibit net carbon dioxide dark fixation. When examined after the photoperiodic state described, long day plants showed approximately three times more water loss over a 10-day period than short day plants. Water loss is similar during light and dark periods for short day plants but long day plants exhibited two times more water loss during the day than at night. The latter plants also lost three and one-half times more water during the light period than short day plants. The water conservation by short day plants is correlated with conditions of high carbon dioxide dark fixation and effects of its related Crassulacean acid metabolism on stomatal behavior.

*Kalanchoe blossfeldiana* is a member of the Crassulaceae consisting mostly of fleshy herbs and subshrubs referred to generally as succulents. Such plants are normally found in arid places, but mainly in South-central Asia, the Mexican highlands, South Africa, and the Mediterranean regions. In the United States they are primarily located in arid regions of the West and Southwest (7). *Kalanchoe blossfeldiana* is a native of Madagascar in the Indian Ocean (1).

Evaporation is high in arid regions due to high temperatures, wind, high vapor pressure deficits in the air, and sandy soils. These conditions present problems of water conservation for plants inhabiting such areas. Succulents have several well known morphological features that aid in water conservation, particularly during short, hot days of little rainfall, as in late winter and early spring (14). A physiological feature associated with many succulents is the night opening of stomates and their closure during the daytime (8, 9), which is related to night accumulation of organic acids and their disappearance during the day—referred to as Crassulacean acid metabolism (2, 10). This phenomenon is attributed by some to have an adaptive significance for conservation of water and CO₂ in succulents (6, 16).

A further examination of this theory of water conservation in succulents would be by study of a plant species which exhibited alternate states of CO₂ dark fixation and its related CAM.³ The Tom Thumb variety of *Kalanchoe blossfeldiana* represents such a plant. In response to long photoperiods, Tom Thumb plants remain vegetative, show relatively little succulence, and show no net CO₂ dark fixation. On short photoperiods they develop thicker leaves, flower prolifically, and exhibit extensive net CO₂ dark fixation (4, 17, 18). During preliminary studies on CO₂ dark fixation, we observed that plants treated on short photoperiods in complete nutrient solution also required less frequent replenishment of solution than plants treated on long photoperiods. This suggested to us a conservation of water uptake by short day plants even with an abundant and continuous source of water available.

The object of this study was to treat Tom Thumb plants in nutrient solution with contrasting photoperiods and correlate the phenomenon of CO₂ dark fixation with the tendency for water conservation. Where such fixation is observed, we assumed it to be directly related to CAM as determined in previous studies (3, 5, 13, 15, 17).

MATERIALS AND METHODS

Culture and Treatment of Plants. Plants used in this study were the Tom Thumb variety of *Kalanchoe blossfeldiana* v. Poeln. Initially, all plants were vegetative and relatively similar in weight, leaf area, and leaf thickness. They were grown as rooted plants in black polyethylene containers filled with a complete nutrient solution (Table I) and fitted with styrofoam lids. Plant stems were confined by polyfoam retaining collars and the root systems were aerated continuously.

Plant systems were placed in an experimental growth chamber the top of which was fitted with a glass-bottomed, copper-lined water bath filled with 10 cm of water in continuous circulation. The water adequately screened out heat from four 150-w incandescent lamps providing light intensity of 1200 ft-c at the plant level in the chamber. Air temperature within the cuvette and growth chamber when closed off for treatments was 23 ± 3 C.

One set of plants was maintained in a growth chamber for 6 weeks on short photoperiods of 8 hr of light and 16 hr of darkness. Another set of plants was treated in another growth chamber on simulated long photoperiods (with short dark periods) consisting of 8 hr of light and 16 hr of darkness, interrupted in the middle by 15 min of incandescent light from the 150-w lamps. Thus the photoperiods were short day (6L/12D/6L) and long day (6L/5.87D/0.25L/5.87D/6L). Photoperiods were given in this manner because it was demonstrated that light quantity (duration × intensity) affected the degree

³ Abbreviation: CAM: Crassulacean acid metabolism.
of CO₂ dark fixation (19), and to examine water loss during essentially equal light-dark periods. After 6 weeks of such photoperiodic treatments, the short day plants were flowering prolifically and had developed thicker, more succulent leaves than the long day plants, which remained in a vegetative state. Having responded in this manner to photoperiodic treatments, the plants were considered to be in an appropriate state for measurements of CO₂ dark fixation and water conservation.

**Determination of Capacity for CO₂ Dark Fixation.** Prior to measurements on water conservation capacity of the long and short day treated plants, the CO₂ dark fixation pattern was determined for one plant of each set using an infrared gas analyzer (Beckman Model 215). The apparatus used to measure CO₂ exchange by the plants was a standard open continuous flow system. The plant examined was enclosed in a 2-liter transparent Plexiglas chamber made airtight except for inlet and outlet portals situated at the top and bottom of the cuvette, respectively. Compressed air at 340 μl/l CO₂ concentration was directed through the open system at 425 ml/min as monitored on a predictability flow meter. The flush rate was approximately 1 volume of free air space per 2 min. Pressure in the system was maintained at 1 cm of Brodies solution on the manometer. The CO₂ concentration measured with the infrared gas analyser was monitored with a photovolt potentiometric recorder and reported as the difference between the CO₂ entering the system from the compressed air cylinder and the net CO₂ leaving the plant system from the Plexiglas chamber.

**Determination of Water Conservation.** Water conservation by the plants was simply determined by measuring the amount of water loss from the entire plant shoot system through transpiration. This was accomplished by weighing the complete plant system, including the container and its nutrient solution, at the beginning and end of three successive test periods over 10 days. The container was brought up to its original weight with nutrient solution prior to the start of a new test series. For a determination of day-night water loss, measurements were also made prior to and after each light period for 2 successive days in the second test series.

Plants were also weighed separately at each test interval to establish the gram fresh weight for the period. Water loss is reported on a gram fresh weight basis since water exchange was associated with the entire shoot, including inflorescences, and not just the leaves. Differences in stomatal number and behavior as well as development of thicker leaves and inflorescences on a short photoperiod made comparisons by leaf area impractical. Schwabe (12) established that additional succulence related to leaf thickening is due to added layers of mesophyll cells in the leaves of these plants rather than to extensive increases in water content. Weight comparisons more readily accounted for such differences as they arose. Weighing was done to 0.01 g on an Ohaus triple beam balance for gross fresh weight determinations and on a Metler microbalance for measurements on dissected plant tissues. A control system of plant container and its solution with no plant was also weighed at the same time as other regular plant systems to determine water loss by evaporation during testing periods. In general, water conservation was reflected by the capacity of plants to restrict water loss by transpiration from the shoot system and its subsequent replacement through the root system from the nutrient solution.

**RESULTS**

**Capacity for Carbon Dioxide Dark Fixation.** The CO₂ dark fixation pattern for one of the long and short day plants after 6 weeks of photoperiodic treatment is presented in Figure 1. This pattern of CO₂ exchange for both plants is supported by previous studies (4, 18) and reflects the degree of CAM that takes place in the light-dark period in both sets of plants.

As shown in Figure 1, the short day plant exhibits a rapid net CO₂ evolution in the early hours of the dark period followed by a pronounced net CO₂ fixation which reaches a maximum at 11 to 12 hr after darkening. Such CO₂ uptake is directly related to the formation of organic acids of which malic is predominant (5, 15, 17). The final hours of darkness for the short day plants are characterized by a rapid decline in net CO₂ fixation and eventual CO₂ evolution. When lights come on at the end of the dark period, the photosynthetic CO₂ uptake is shallow for the short day plant and typically shows a period of CO₂ evolution that we consider to be an overshoot of the deacidification process which occurred at this time.

The long day plant exhibits no net CO₂ fixation throughout the dark period and its fixation pattern is particularly distinguished from the short day plant by the rapid and pronounced photosynthetic CO₂ fixation during the light period.

**Extent of Water Conservation.** Table II shows water conservation data over the first 5 days and clearly indicates that on a daily and over-all basis the plants treated on long photo-

---

Table 1. Composition of Full Strength Nutrient Solution in which Plants Were Grown

<table>
<thead>
<tr>
<th>Salt</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH₂PO₄</td>
<td>2 x 10⁻³</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>2 x 10⁻³</td>
</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td>3 x 10⁻³</td>
</tr>
<tr>
<td>KNO₃</td>
<td>2 x 10⁻³</td>
</tr>
<tr>
<td>NaCl</td>
<td>1 x 10⁻³</td>
</tr>
<tr>
<td>H₂B₄O₇</td>
<td>4.6 x 10⁻⁵</td>
</tr>
<tr>
<td>MgCl₂·4H₂O</td>
<td>9.2 x 10⁻⁶</td>
</tr>
<tr>
<td>ZnSO₄</td>
<td>8.1 x 10⁻⁷</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>2.9 x 10⁻⁷</td>
</tr>
<tr>
<td>Na₂MoO₄</td>
<td>5.7 x 10⁻⁷</td>
</tr>
<tr>
<td>Sequestrene Na·Fe</td>
<td>1 x 10⁻⁴</td>
</tr>
</tbody>
</table>

---

*Fig. 1. Carbon dioxide dark-fixation patterns for long and short day plants of Kalanche blossfeldiana var. Tom Thumb, as measured with an infrared gas analyser after 6 weeks of photoperiodic treatments.*
RESULTS

Table II. Water Loss in Kalanchoe blossfeldiana var. Tom Thumb following 6 Weeks of Photoperiodic Treatments

<table>
<thead>
<tr>
<th>Test Series</th>
<th>Short Day Plants</th>
<th>Long Day Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series I. Initial 5 days (avg water loss/g fresh wt-day)</td>
<td>10.3 ± 0.8</td>
<td>29.9 ± 0.8</td>
</tr>
<tr>
<td>Series II. Middle 2 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In light periods (avg water loss/g fresh wt)</td>
<td>9.95 ± 0.9</td>
<td>35.3 ± 1.6</td>
</tr>
<tr>
<td>In dark periods (avg water loss/g fresh wt)</td>
<td>9.65 ± 0.2</td>
<td>16.8 ± 0.3</td>
</tr>
<tr>
<td>Entire 2-day period (avg water loss/g fresh wt-day)</td>
<td>9.7 ± 0.8</td>
<td>26.3 ± 0.5</td>
</tr>
<tr>
<td>Series III. Final 3 days (avg water loss/g fresh wt-day)</td>
<td>8.8 ± 0.5</td>
<td>23.0 ± 1.3</td>
</tr>
<tr>
<td>Entire 10-day period (avg water loss/g fresh wt-day)</td>
<td>9.7 ± 0.7</td>
<td>27.1 ± 0.7</td>
</tr>
</tbody>
</table>

periods have 3 times the water loss of plants treated on short photoperiods. Water loss by the plants through transpiration is comparable to water uptake by the roots as indicated by the similar amount of water replacement required at the end of the test period.

Similar determinations of water loss over a 2-day period in series 2 and another consecutive 3-day period (series 3) is also shown in Table II. Summary data for all three series presented in Table II shows that over the complete 10-day period, the average water loss was fairly consistent among both long and short day plants but approximately 3 times greater for long day plants in general.

Series 2 was conducted to determine the time during the 24 hr of treatment when most of the water loss occurred. Data of Table II shows that while water loss from the short day plants is about the same for the light and dark periods, the long day plants lose twice the amount of water during the day as at night and three and one-half times the amount of water during the daytime compared to plants treated on short days.

DISCUSSION

The Tom Thumb variety of Kalanchoe blossfeldiana was selected for this study of water conservation by a succulent plant because of its contrasting responses to CO₂ dark fixation under photoperiodic treatment. It was expected that because of stomatal effects, plants which exhibited pronounced net CO₂ dark fixation and its related CAM would show a decided tendency for conservation of available water compared to plants of the same variety which did not show this phenomenon. This expectation was fulfilled.

The important consequence of photoperiodic treatments for Tom Thumb in this study is that extensive net CO₂ dark fixation occurs only for the plants treated on short days. This results in the formation of large amounts of organic acids in the leaves which apparently causes stomates to open during the dark period due to a lowering of the pH and subsequent increase in the osmotic value or amphoteric swelling of colloids, according to Nishida (9). Decarboxylation of these acids in the light then reverses the process controlling stomatal size and results in their closure during the daytime. The opposite stomatal response must prevail in long day plants if the relationship to CO₂ dark fixation and accumulation of organic acids is consistent. This prevalence that for long day plants, the stomates are predominantly closed at night and open in the daytime. Nishida (9) reported this situation for succulent plants not showing CO₂ dark fixation. Schwabe (11) also demonstrated open stomates at night in short day plants and closed stomates in long day plants of Chrysanthemum, suggesting that photoperiod alone may control stomatal behavior.

The influence of a particular CO₂ dark fixation pattern and its related stomatal behavior on water conservation in the two sets of plants is considerable. Although both sets of plants had abundant water to utilize continuously, only plants treated on long days exhibited excessive water loss by transpiration and subsequent uptake by the roots. Daytime loss of twice the nighttime loss and three and one-half times the daytime water loss of short day plants undoubtedly reflects the opened stomate condition of long day plants in the light period. This also resulted in a characteristically high photosynthetic CO₂ fixation as shown in Figure 1. Short day plants may have an equally high CO₂ fixation in light, but such CO₂ apparently originates internally from decarboxylated organic acids formed during the previous dark period (5, 16). Uptake of atmospheric CO₂ would not be necessary and the resulting CO₂ fixation pattern is quite shallow compared to long day plants as shown in Figure 1. The lower water loss at night in short day plants is additional evidence of water conservation in spite of open stomates and extensive CO₂ dark fixation at this time. The response could be due to increased diffusion resistance of water vapor in mesophyll cells resulting from lower water potential that must develop during organic acid formation in the mesophyll.

The water conservation observed in this species as a result of induced CO₂ dark fixation and its related CAM is not considered by us to be associated with a capacity for water retention as in the capacity to restrict transpiration and subsequent water uptake by the plants. This water conservation could be important under xerophytic conditions for the Tom Thumb variety of Kalanchoe blossfeldiana. On relatively short, hot days under conditions of little available soil water and appreciable wind, the lessen tendency for short day plants to take up and transpire water while retaining a high photosynthetic capacity would be a considerable advantage. High rates of CO₂ dark fixation and consequent formation of organic acids on short days would assure appropriate stomatal conditions for these arid conditions. Conversely, for Tom Thumb plants on long days such conditions would represent very unfavorable circumstances for the species. With no diurnal formation of organic acids from CO₂ dark fixation, stomates would possibly remain open during the day developing water stress conditions which the plants might not survive over a long period. An unbalance of water economy within the leaf alone would not necessarily affect the stomatal changes of this succulent as Nishida (9) reported for Bryophyllum.

The photoperiodic limitation imposed on the Tom Thumb variety of Kalanchoe blossfeldiana, therefore, would appear to give it less adaptive advantage in terms of water conservation than plants which exhibit extensive net CO₂ dark fixation and CAM on both long and short days such as the Feuer Blute variety of K. blossfeldiana (18).

LITERATURE CITED

WATER CONSERVATION IN KALANCHOE