FLOWER FORMATION IN *EUCHARIS AMAZONICA* LINDEN EX PLANCHON

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Abstract

Flower formation in *Eucharis amazonica* Linden ex Planchon is induced when plants are exposed to 4 weeks at 27°C followed by 21°C. Eighty to 100% of the plants flower after 90 to 95 days at 21°C.

The bulb has one terminal growing tip. At 21°C it changes from vegetative to generative, and a vegetative lateral growing tip is being formed. All flowers of the inflorescence (two helicoid cymes) are completed after 28 days at 21°C. The vegetative lateral growing tip is morphologically complete after 19 days at 21°C. After 56 days at 21°C it can be induced to flower formation, if plants are exposed again to 4 weeks at 27°C, followed by 21°C. After 90 to 95 days at 21°C the plants will then flower again. Generally, a temperature drop of about 6°C is a prerequisite for floral induction. The low temperature should, however, not be too low (13°C), to avoid growth arrest. Temperature-controlled flower formation in *Eucharis amazonica* allows the production of 4 cut flowers per plant per one year.

Flower formation is also induced after drying out to wilting and then rewatering the plants. As a result, only 25% of the plants flower, 95 days after withholding water. Stress is thought to be the common cause for the induction of flower formation after a change in temperature or after wilting.

Introduction

Flower formation in *Eucharis amazonica* Linden ex Planchon (Meerow and Dehgan, 1984) is induced when leafy plants with a bulb diameter of at least 35 mm, growing at 21°C, are exposed to 27°C during 4 weeks, and next transferred back to 21°C. The plants flower 90 to 95 days after the end of the 27°C treatment (Van Bragt and Sprenkels, 1983a). Deba and Meyers (1973) and Deba (1978) found no anatomical changes in the vegetative apical meristem until 2 weeks after the high temperature treatment. From then on the apex developed into an inflorescence with the subsequent development of a vegetative lateral growing tip, which eventually could be induced to flower formation. Deba and Meyers (1973) stated that a 28-day minimal rest period was optimal between last flower removal and initiation of a new flowering cycle. Van Bragt and Sprenkels (1983b), however, showed that treatment at 27°C followed by 21°C induced a new flowering cycle when applied to plants with their first flower open. Baily (1917) stated that flowering of *Eucharis* was induced if plants were kept dry until near wilting for a period of 1 month and then thoroughly watered.

This paper reports SEM examinations of the developmental stages of flower formation. The results generally confirm the observations of Deba (1978) and show that flower formation does not start during the 4 weeks'

exposure to 27°C, but only after the subsequent transfer to 21°C.

The paper also reports experiments designed to elucidate further the effect of temperature on flowering.

Finally the paper describes flowering of plants which had been kept dry until near wilting, followed by thoroughly soaking in water.

Material and methods

Developmental stages of the inflorescence and the lateral bud

Plant material

Potted plants with 4 to 5 leaves were grown on benches in a greenhouse at 21°C (both day and night). The plants were shaded to prevent leaf injury. For flower formation, plants were exposed for 4 weeks to 27°C, next transferred to the greenhouse at 21°C (Van Bragt and Sprenkels, 1983a).

Preparation of plant material for SEM examination

Examinations were carried out on plants from 2 weeks before the 4 weeks' 27°C treatment until 8 weeks after this treatment. Twice a week 3 plants were dissected. Growing tips were fixed for 4 hours in 5% glutaraldehyde, next for 1 to 2 hours in 1% osmiumtetroxide. Water and osmiumtetroxide were removed in a graded (10 to 100%) series of ethanol. The tips were critical-point dried with CO_2 as the transient fluid. The dried specimens were attached to SEM stubs with conductive carbon cement and coated with gold and observed in a Jeol JSM-35C SEM (Technical and Physical Engineering Research Service, TFDL, Wageningen, The Netherlands) at 15 KV. The experiments were carried out twice.

Effects of temperature treatments on flowering

Using vegetative plants growing at 21°C (day and night), experiments 1, 2 and 3 were designed to further establish the influence of changes in temperatures on flower formation. For each experiment (20 plants per treatment) the percentage of flowering plants was recorded.

Experiment 1

Plants were exposed to 27, 25 or 21°C for 1 to 5 weeks, then transferred back to 21°C.

Experiment 2

Plants were exposed for 4 weeks to 27°C, then transferred to 17, 21, 25 or 27°C.

Experiment 3

Plants were exposed for 4 weeks to 13, 17 or 21°C, then transferred to 21°C.

A fourth experiment was designed to establish from when the newly formed vegetative lateral bud could be turned into generative by means of temperature treatment.

Experiment 4

Plants were exposed for 4 weeks to 27°C, next to 21°C. After 4, 6, 8, 12 or 16 weeks at 21°C they were exposed for a second period of 4 weeks to 27°C and again transferred to 21°C. 20 Plants were used per treatment.

Flowering after withholding water

Plants were grown in clay pots on benches in a greenhouse at 21°C (both day and night). The soil mixture was as described earlier (Van Bragt and Sprenkels, 1983a). Thirty control plants were regularly and thoroughly watered. Water was withheld to 60 other plants during 10 days until they were near wilting. At that time the soil suction tension was 0.52 to 0.65 bar (tensiometer). With regular gifts of small quantities of water, this tension was maintained during 14 days. The plants were subsequently soaked in water. Time to flowering and % flowering were recorded.

Results

Developmental stages of the inflorescence and the lateral bud

During the 27°C treatment, the apical meristem remains vegetative, a continuous sequence of leaf formation form a flattened stem tip (Fig. 1a). Therefore, the day of transfer from 27 to 21°C was designated day 0. After the 27°C treatment the apex turned from vegetative into generative and had become convex on day 5 (Fig. 1b). On day 10 the two bracts were visible. On day 14 the two oldest flowers (1 and 2) of the two future helicoid cymes and their bracteoles appeared, on day 21 both second flowers (3 and 4) arose from the axils of the latter bracteoles and on day 28 both third flowers (5 and 6) appeared in the axils of bracteoles 3 and 4. From that moment the inflorescence was complete and consisted of two helicoid cymes, both being either left- or right-rotating (Figs. 1c and 1d). The first flower of the oldest helicoid cyme was complete on day 28 and developed as follows: outer tepals on day 17, inner tepals day 19, outer whorl of stamens day 21, inner whorl of stamens day 23, stigmata lobes day 28 (Fig. 1e). On day 56 the inflorescence emerged in the center of the plant and the oldest flower opened on day 95.

On day 12 a vegetative lateral primordium developed outside the inflorescence, at the base of the oldest bract. On day 19 this primordium showed a developing leaf (Fig. 1f).

Effect of temperature treatments on flowering

Experiment 1

The results are presented in Table 1 which shows that the % flowering plants increased if plants had been exposed to 25 or 27°C during periods of 2 to 5 weeks, and that a high % flowering was found only when 3 to 5 weeks at 27°C was followed by 21°C, a decrease of 6°C.

Table 1. Percentage of flowering plants after 1 to 5 weeks at 21, 25 or 27°C followed by 21°C until flowering.

Temperature treatment	Duration of temperature treatment (weeks)				
	1	2	3	4	5
21°C followed by 21°C	0	5	10	0	10
25°C followed by 21°C	0	25	40	45	20
27°C followed by 21°C	5	45	95	95	95

Experiment 2

The flowering % of plants exposed for 4 weeks to 27°C, then to 27, 25, 21 or 17°C until flowering was 5, 45, 90 and 80, respectively. Thus, a high % of flowering plants was observed after transfer from 27°C to either 21 or 17°C, a decrease of 6 and 10°C, respectively.

Experiment 3

Flowering % was 30, 10 and 5, respectively for plants grown at 21°C and subsequently transferred to 13, 17 or 21°C. It is noted that a low percentage of flowering was obtained after transfer from 21 to 13°C and also after 21 to 17°C. In the first case there is a 8°C difference in temperature (cf. experiment 2: 6°C), but the lowest temperature is 13°C. In the second case the temperature difference is only 4°C.

Experiment 4

Table 2 shows that the first exposure from 27 to 21°C resulted in 85 to 100% flowering. The second exposure also led to flowering, but only if at least 8 weeks at 21°C had passed before the start of the second exposure to 27°C.

Table 2. Percentage of flowering plants after a first exposure for 4 weeks to 27°C, followed by 4 to 16 weeks at 21°C and a subsequent second exposure for 4 weeks to 27°C followed by 21°C until flowering.

Group	Exposures				% Flowering as result of exposures	
	First		Second		First	Second
	27°C to 21°C		27°C to 21°C			
1	all plants, 4 weeks	4 weeks	all plants, 4 weeks	all plants until flowering	85	0
2		6 weeks			100	0
3		8 weeks			90	95
4		10 weeks			85	85
5		12 weeks			90	80
6		16 weeks			95	95

Effect of keeping plants near wilting, followed by rewatering, on flowering

Twentyfive % of the plants flowered after rewatering. Five % of the control plants flowered. The treated plants flowered 71 days after rewatering. Thus, 71 + 10 + 14 = 95 days had passed from the time that water was withheld to the plants, until flowering time.

Discussion

Developmental stages of the inflorescence and the lateral bud

The developmental sequence of the inflorescence is almost similar to the one described for the narrowly related species *Hippeastrum spp.* (Beyer, 1942) and *Amaryllus belladonna* (Hartsema and Leupen, 1939), both having the same type of inflorescence as *Eucharis*. The flowers of the

first helicoid cyme develop a little earlier than their respective partners of the second helicoid cyme. In this way a developmental range arises, according to the flower numbers 1 to 6 in the Figs. 1c and 1d, also resulting in a gradual spreading of the flowering time.

The fact that during the 27°C treatment the terminal primordium remains vegetative indicates that floral induction is not only a result of this high temperature itself, but also of the sudden drop to 21°C.

Our earlier statement that the lateral bud needs a lag time before it can be induced to generative development (Van Bragt and Sprenkels, 1983b) can now be supplemented by the conclusion that this appears to be independent from the morphological stage. Although the lateral bud is formed relatively early after the temperature drop from 27 to 21°C, its leaf primordium remains in a very young stage of development, comparable with the stages in which the terminal bud could be induced to flowering. This morphological similarity between the inducible terminal bud and the non-inducible lateral bud suggests a physiological base for the lag time of the latter.

Besides, Van Bragt and Sprenkels (1983b) reported that after a second induction the lateral bud produces an inflorescence after having developed only one normal assimilatory leaf. As this second 27°C treatment does not influence the development of the first inflorescence, repeated flower production seems to be possible in this species. In general, however, monocotyledonous species in their vegetative cycle produce young leaves continuously, while older leaves die simultaneously. So the question remains whether the plant can endure repeated production of inflorescences together with just a small amount of leaves. In case the older leaves, instead of dying, keep on assimilating for a longer period this will probably be the case. If not, a period of recovery will be necessary to renew the assimilatory apparatus of the plant.

Temperature-controlled flowering

All temperature treatments had one feature in common: they included a step in which the temperature was lowered. This resulted in a high percentage flowering (about 90) if the higher temperature had been applied during a certain time (e.g. 4 weeks at 27°C) and then lowered with 6°C or more (e.g. from 27 to 21°C). The observation that no appreciable flowering occurred in case the lower temperature was 13°C does not come as a surprise. At that temperature the growth of *Eucharis* - native in Colombia and Peru (Rees, 1985) - is seriously reduced.

After a 27 to 21°C treatment, a subsequent 27 to 21°C treatment will induce a second flowering, if at least 8 weeks at 21°C have been allowed before this subsequent treatment is given. In practice this means that a second 27 to 21°C treatment can be given when the inflorescence - result of the first treatment - is visible in the center of the plant.

Flowering after withholding water

This treatment results in a low percentage (25) of flowering plants. Experiments with drying until near wilting were carried out at 21°C. This allows to deduct the time of floral initiation in these plants. SEM observations learned that at 21°C it took 95 days from floral initiation to flowering. From these data it is concluded that in our experiments floral induction occurred soon after the water was withheld, from that time it took 95 days until flowering.

Each of the conditions: (1) a sudden and appreciable drop in temperature, and (2) withholding water may have exerted their effects on floral

initiation because of one common cause: they induced "stress" in the *Eucharis* plant.

It remains, however, to be explained why control plants occasionally flower, and why flowering percentages vary from 0 to 100 after some treatments. We never observed aborted flowers in the bulb. Thus, it remains to be elucidated which plant factors other than bulb size are determinants of the induction of flower formation in *Eucharis*.

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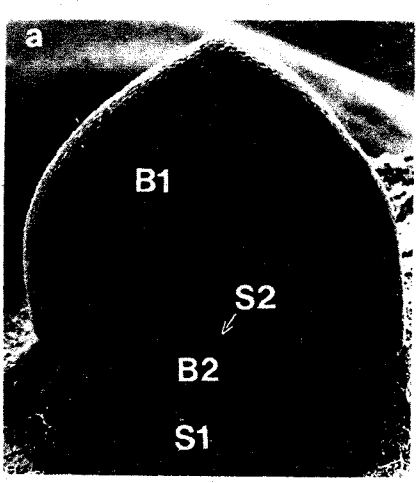
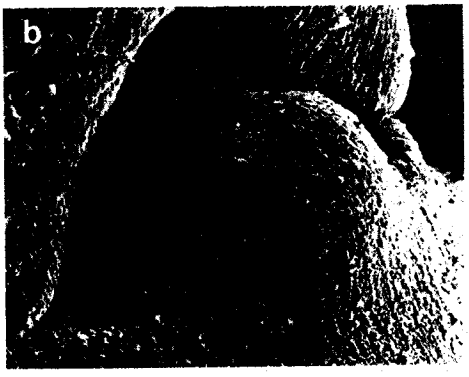
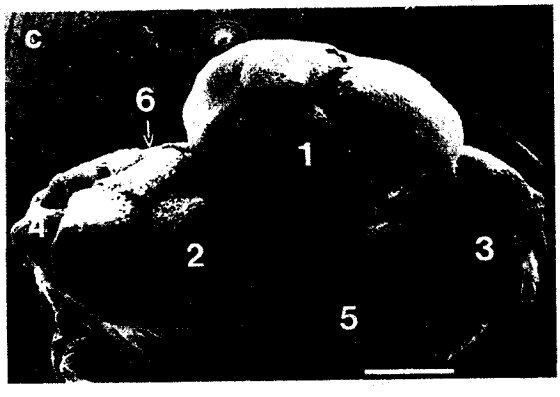


1a - The vegetative apex. A leaf blade (B1) with its sheath (S1) surrounds a younger leaf blade (B2) with sheath(S2) Between the latter two a flattened stem tip is present (not visible) (32 x).

Figure 1. Developmental stages of the inflorescence and the lateral bud of *Eucharis amazonica* Linden ex Planchon.

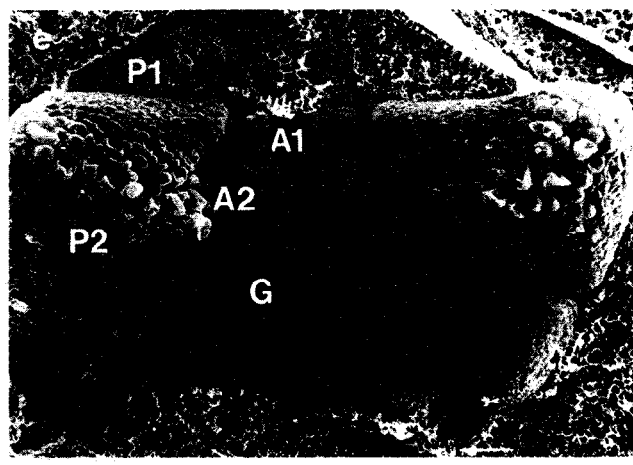


1b - The generative apex. The youngest leaf remains unfinished. Its sheath is not formed and the stem tip turns into a conves shape (130 x).



1c The complete inflorescence. Two helicoid cymes of three flowers (1-3-5 and 2-4-6 respectively) are visible. The bracts and bracteoles are removed (18 x).

1d - The same inflorescence as in Figure 1c. The flowers 4 and 6 are not visible (13 x).



1e The flower after 28 days. P1 and P2 = outer and inner whorl of tepals, respectively, A1 and A2 = outer and inner whorl of stamens, respectively, G = stigma lobes. Both the outer and one of the inner tepals have been cut off (90 x).

1f The lateral primordium, at the base of the oldest bract (Br1), has formed a (vegetative) leaf blade (B) with its sheath (S). Behind the partially cut off bract a part of the inflorescence is visible (36 x).