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Promotion of Flowering in *Brassica campestris* L. cv Ceres by Sucrose

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ABSTRACT

Flower initiation of the quantitative long-day plant *Brassica campestris* cv Ceres was earlier and at a lower final leaf number when sucrose was added to the medium in which plants were grown in sterile culture. The optimal concentration of sucrose was 40 to 80 millimolar. This flower-promoting effect of sucrose was not osmotic, as mannitol, sodium chloride, and polyethylene glycol were not effective at equal osmotic potentials.

Seedlings grown heterotrophically after treatment with 4-chloro-5-(dimethylamino)-2-phenyl-3-(2H)-pyridazinone to prevent chlorophyll accumulation were also induced to form flower primordia earlier as the sucrose concentration in the medium was increased up to 80 millimolar. Inclusion of 4 millimolar sodium nitrate in the culture medium of green plants did not reduce the flower-promoting effects of sucrose but delayed initiation in plants grown without added sucrose.

Removal of CO_2 during a single main or supplementary light period, or both, greatly reduced flower initiation. It is concluded that sucrose may be an important controlling factor determining floral initiation in *Brassica*.

Under natural conditions the daylengths that control flowering of many plants occur at intensities usually sufficient to saturate photosynthesis, and both high and low irradiance reactions may proceed simultaneously. In SD plants one major control of flowering by daylength operates through the low intensity reactions of phytochrome (2, 19), but in many LD plants the intensity given must be higher than that usually required to saturate typical phytochrome responses and may approach that needed to saturate photosynthesis (2, 15). These HIR[1] have been ascribed to a special action of phytochrome as well as to the activity of a blue-absorbing pigment (2, 19). The HIR effects of daylength have been separated from those of simultaneous photosynthesis in barley (11) by treating plants with pyridazinone herbicides to prevent Chl accumulation. There may, however, be a need for the products of the dark reactions of photosynthesis, such as sugars, to interact with phytochrome and HIR reactions. CO_2 is needed during a photoinductive light treatment in several LD plants (1, 8, 14, 16), and sugar feeding after CO_2 removal at least partially restored the original level of flowering in some cases. Chemical inhibitors of photosynthesis such as DCMU have also reduced flower initiation in some LD plants (2, 15, 19).

The earlier concepts of the importance of carbohydrate supply for flower induction (12, 13) have been revived by Grainger (10). A partial correlation between flower induction and an increased soluble carbohydrate concentration in the stem apex has been found in cauliflower (6, 17) and *Sinapis* (3, 4). Direct addition of sucrose to the medium in which stem tips of the LD plant *Sinapis* were grown in sterile culture induced flowering under SD (5), suggesting that under some conditions floral initiation may be causally dependent on carbohydrate supply.

The quantitative LD plant *Brassica campestris* cv Ceres was selected for its small size, seedling sensitivity to daylength, and rapid response (9). Floral initiation is earlier, the higher the photon flux density under which plants are grown, and both HIR and phytochrome responses are involved (7). A linear relationship between the reciprocal of daylength and time to floral initiation and the equal effectiveness of fluorescent and incandescent light given at equal PAR, suggested that photosynthesis plays a quantitative role in the induction of flowering (8).

These experiments were designed to determine whether increasing the carbohydrate supply of either green plants or plants treated with Sandoz 6706 to prevent Chl accumulation would increase the quantitative effects of daylength and photon flux density on flower initiation in *Brassica* seedlings. The effect of reducing the carbohydrate supply was studied by using CO_2-free air to restrict the rate of photosynthesis.

MATERIALS AND METHODS

Plant Material. Seeds of *Brassica campestris* L. cv Ceres (9) were dusted with Orthocide to prevent damping-off and sown and covered in damp vermiculite (particle size 25 mm diameter) in plastic pots 7 cm in diameter and 7 cm deep at a density of from 20 to 30 seedlings per pot. Seeds were kept at a low light intensity (30 μmol m^{-2} s^{-1} PAR) under an 8-h daylength for 2 d to prevent surface drying of the vermiculite, and then placed under the desired light treatment. Cotyledons opened on the 4th d after sowing.

CO_2 Removal. In experiments on the effects of CO_2 removal, plants were grown in vermiculite for 4 d under an 8-h daylength. On the 5th d they were placed in glass tanks through which a stream of normal or CO_2-free air was passed at an initial rate of 200 L·h^{-1} followed by a rate of 50 L·h^{-1}. Two wash bottles containing 10% w/v KOH solution were used to scrub CO_2 from the air and a bottle of saturated $Ba(OH)_2$ solution was used to indicate that CO_2 was removed. Plants were kept under normal or CO_2-free air for either the 8 h of the main light period and/or during 16 h supplementary irradiation from a Phillips HP1T 2000 w mercury halide lamp at a photon flux density of 300 μmol m^{-2} s^{-1} (see Table IV for treatment combinations). After the single LD treatment, plants were returned to 8-h SD until dissection 11 or 12 d after sowing. The experiments were repeated three times.

Sterile Culture. For sterile culture, two seeds were dipped in 3% v/v H_2O_2 for about 5 s and sown on the surface of 2 ml of heat-sterilized 0.6% w/v agar medium in test tubes 15 cm long by 8 mm diameter. The desired amount of sucrose or other sugar

[1] Abbreviations: HIR, high irradiance response; Sandoz 6706, 4-chloro-5-(dimethylamino)-2-phenyl-3-(2H) pyridazinone.

or osmoticum was added to the agar before autoclaving. Osmotic potentials of the agar medium were determined with a Wescor vapor pressure osmometer for four samples of each type used.

Effects of Sucrose on Final Leaf Numbers. A decrease in the number of leaves produced before an influorescence is often used to distinguish between a specific promotion of the rate of flower initiation and a promotion of the rates of both leaf and flower initiation through a general increase in growth (2). A separate series of experiments were carried out because in the previous experiments plants died before a conclusive assessment of final leaf numbers could be made. Plants were cultured on 4 ml of agar medium in tubes 23 mm in diameter and 15 cm long, two plants per tube as before. The agar was made up with either one-eighth or one-quarter strength Hoagland solution and contained either 0, 40, 80, or 120 mM sucrose. Plants were dissected after 14 d growth for the determination of flower initiation and after 35 d for counting the final leaf number.

Chl Bleaching. For treatments where Chl accumulation was prevented by the action of Sandoz 6706, 0.1 ml of a 100-mM solution of Sandoz 6706 in acetone was added to each test tube and the acetone allowed to evaporate to dryness before adding the agar medium and autoclaving. The final concentration of Sandoz 6706 in the medium was 5 μM. Previous experiments had shown that this was the lowest concentration that consistently inhibited Chl accumulation completely. Plants on vermiculite and on agar medium were deprived of nutrient mineral salts to restrict plant size, except in experiments on the effect of nitrogen nutrition when sodium nitrate was added to an agar medium to give a final concentration of 4 mM.

Growing Conditions. Plants were grown in controlled environment cabinets at 25°C for all treatments and the standard light conditions were provided by 8 h daily illumination from cool-white VHO Sylvania Power Tube fluorescent lights at 300 μmol m^{-2} s^{-1} PAR. Flower initiation was determined 10 to 13 d after sowing by examination of the apical meristems of samples of 20 to 30 plants under the dissecting microscope. The number of flower buds or primordia was counted and SE number were calculated.

RESULTS

Effect of Sucrose at Different Photon-Flux Densities. Green plants grown in vermiculite under a 12-h daylength had a higher percentage of flowering after 12 d growth the higher the photon flux density, from 36 to 400 μmol m^{-2} s^{-1} PAR (Fig. 1). When plants were grown on agar containing 80 mM sucrose however, floral initiation was just as rapid at low photon flux densities as at high. Inhibition of Chl accumulation by Sandoz 6706 treatment (bleached plants) did not prevent this effect of sucrose in promoting floral initiation. Hypocotyl length of both normal green and bleached plants was inversely related to photon flux density (Table I).

Effect of Sucrose at Different Daylengths. An increase in daylength increased the percentage flowering and mean number of flower buds for green plants grown on plain agar (Fig. 2). Addition of 80 mM sucrose to the medium increased flowering of green plants grown under 8 and 12 h daylengths, but the effect was less under a 16- or 24-h daylength (Fig. 2). Similar promotion of flowering by addition of sucrose to plants grown under short daylengths were obtained in other experiments. Addition of nitrate reduced flowering in the water-agar treatments and the promotion of flowering by addition of sucrose was even greater (Fig. 2).

In other experiments, lengthening the daylength did not significantly alter the flowering of bleached plants but addition of 80 mM sucrose promoted flowering of plants grown under both 8 and 16 h daylengths.

Total dry weights of green plants were increased by about 1 mg by lengthening the daylength and/or adding sucrose to the medium. The dry weight of bleached plants was little affected by daylength, but an increase in sugar concentration from 1 to 80 mM increased the dry weight by about 1 mg per plant.

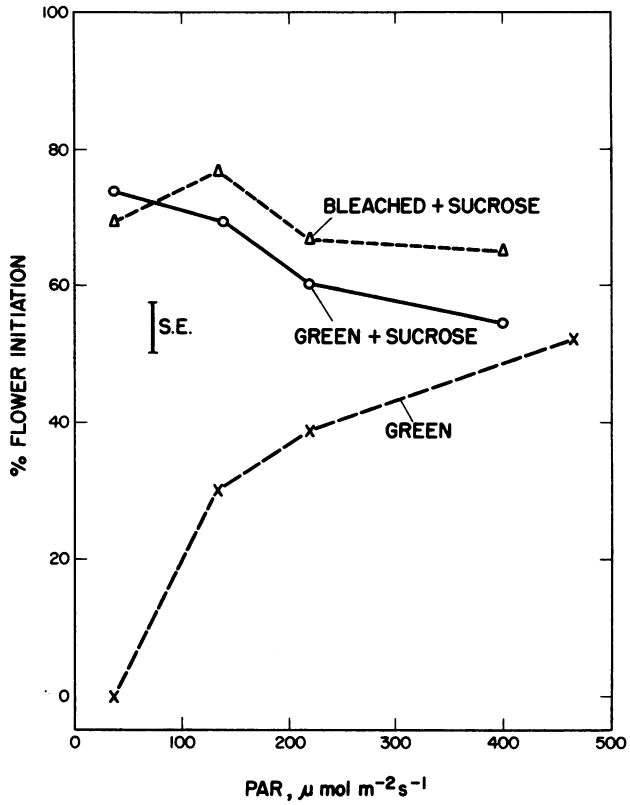

FIG. 1. Effect of photon flux density on % flower initiation of *Brassica* grown on agar containing 80 mM sucrose (O——O, green plants; Δ- - -Δ, bleached plants) or on vermiculite (×- - -×, green plants). Plants grown for 12 d under a daylength of 12 h. Points are means from approximately 15 plants.

Table I. *Effect of Photon Flux Density on Hypocotyl Length (mm) of Brassica Grown on 80 mM Sucrose for 11 Days under a 12-Hour Daylength*

	Photon Flux Density			
	36	125	200	400
	μmol m^{-2} s^{-1}			
Green plants	14.2[a]	7.0	9.6	4.9
Bleached plants	11.7	7.2	5.3	5.1

[a] SE derived from analysis of variance = ±0.68; n = 15.

Effect of Sucrose Concentration. Green plants and bleached plants were grown under an 8-h daylength on agar containing sucrose concentrations varying from 0 to 80 mM (Fig. 3). For the green plants the percentage flower initiation after 12 d growth increased over the range 10 to 80 mM. For bleached plants no floral initiation was obtained below a concentration of 20 mM sucrose. The percentage initiation then increased with further increases in sucrose concentration up to 80 mM (Fig. 3). A concentration of 160 mM was supraoptimal for flowering of both green and bleached plants. Plant dry weights increased with increasing sucrose concentrations from 0 to 80 mM for both green and bleached plants (Fig. 4).

Effect of Sucrose on Final Leaf Numbers. Addition of 40, 80,

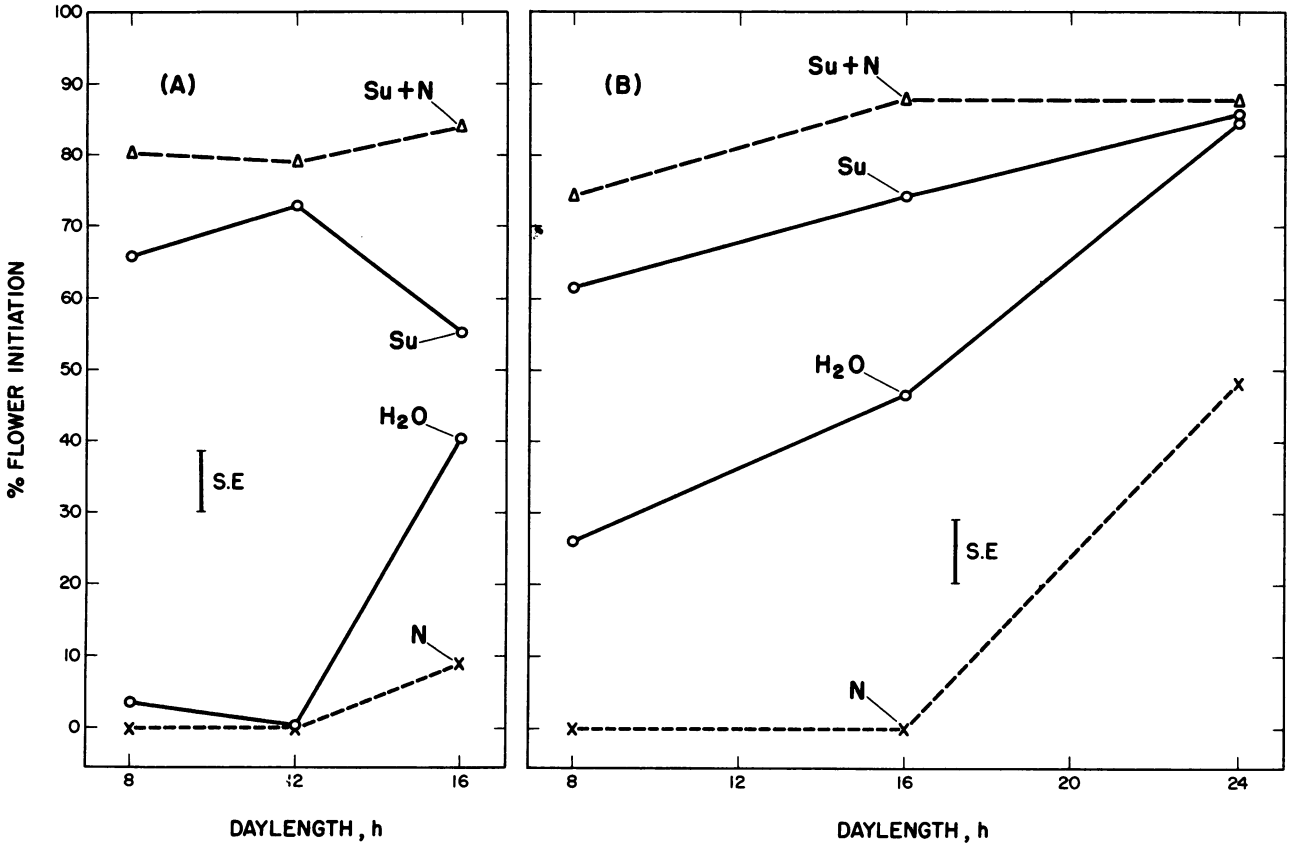

FIG. 2. Flower initiation in *Brassica* as affected by 80 mM sucrose and 4 mM $NaNO_3$ at different daylengths. Green plants grown for 12 d at 25°C. Plain agar medium (O——O H_2O), 80 mM sucrose (O——O Su), 4 mM $NaNO_3$ (×- - -× N), and 80 mM sucrose + 4 mM $NaNO_3$ (Δ- - -Δ Su + N). Each point is a mean from 30 plants. A, Daylengths of 8, 12, 16 h; B, daylengths of 8, 16, 24 h.

or 120 mM sucrose to an agar medium containing one-eighth or one-quarter strength Hoagland solution progressively increased the percentage flower induction. The final leaf number was higher for plants grown on one-quarter strength Hoagland solution than for those grown on one-eighth strength and was inversely related to the concentration of sucrose (Table II).

Osmotic Effects. Green plants were grown for 10 d under an 8-h daylength on agar containing 80 mM sucrose (−310 kPa osmotic potential) or NaCl, PEG, or mannitol of approximately equal or double this osmotic potential (Table III). Plants grown on water-agar remained vegetative while sucrose-treated plants initiated flowers even under the 8-h daylength. NaCl and PEG did not promote flower initiation and mannitol gave only a very low level of stimulation (Table III). The experiment was repeated using 40 and 80 mM sucrose and 20, 40, and 80 mM mannitol, with similar results.

Effects of CO_2 Removal. In normal air, a single extension of the 8-h main light period by 16 h supplementary high intensity light significantly promoted floral initiation (Table IV). When CO_2 was removed during both the main and supplementary light periods or during the supplementary light period alone, the percentage of flower initiation was significantly reduced. An intermediate level of flower initiation was obtained when CO_2 was removed only during the main light period preceding 16 h supplementary light.

DISCUSSION

Sucrose was taken up from the medium, as shown by the increase in total plant dry weights of bleached plants where, in the absence of photosynthesis, the medium was the only source of carbon (Fig. 4). Sucrose, at an optimal concentration of about 80 mM, also promoted flowering of the quantitative LD plant *Brassica* grown under SD conditions. This effect of sucrose on flower initiation has previously been reported for the very similar cruciferous LD plant *Sinapis alba* (5). The lowering of the final leaf number by the addition of sucrose to the medium (Table II) shows that the effect of sucrose was specifically on flower induction rather than simply through a general promotion of growth.

Treatments that lowered the total daily photosynthesis, such as reductions in light intensity (Fig. 1), shortening of the daylength (Fig. 2), and reduction in the CO_2 supply (Table IV) reduced flowering of green plants. Prevention of photosynthesis by treatment with Sandoz 6706 also lowered the level of flowering obtained for a given concentration of sucrose as compared with green plants (Fig. 3). Phytochrome activity was probably little affected by Sandoz treatment (11) as hypocotyl elongation was inversely related to photon flux density in bleached as well as green plants (Table I). The level of flowering was at least partially restored when a low level of daily photosynthesis was compensated by the incorporation of sucrose in the medium (Figs. 1–3), so that the effect of reduced photosynthesis on lowering the flowering response was probably related to the reduced level of carbohydrates. The flower-promoting effect of sucrose was not simply caused by the more negative osmotic potential of the medium, as other nonpenetrating or penetrating osmotica had no significant effect on flowering (Table III). The effects of NaCl on reducing growth may have included toxicity.

A metabolite such as sucrose has so many effects that there are many possible mechanisms by which floral initiation could be hastened. The simplest explanation is probably a modification of the carbohydrate/nitrogen ratio hypothesis of Klebs (2, 12). If

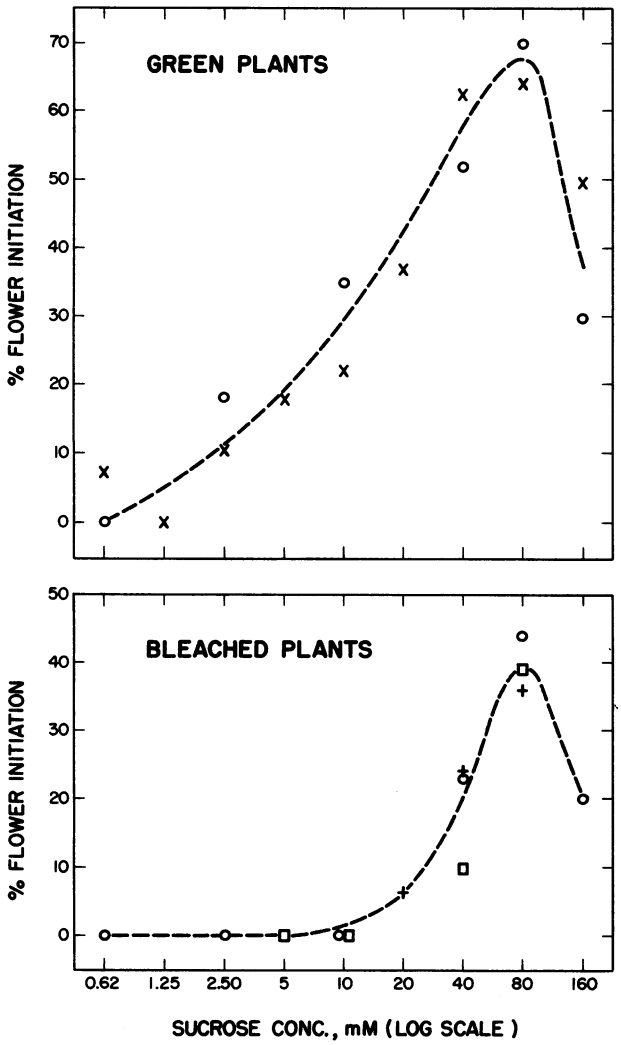

FIG. 3. Effect of sucrose concentration on flower initiation of *Brassica* grown under an 8-h daylength at 25°C for 12 d. Upper green plants, lines hand drawn through points for two replicate experiments (O, ×) each point a mean from 20 to 30 plants, mean value for −Su control = 1.5%. Lower, bleached plants, data from three experiments (O, □, +), mean value for −Su control = 0%.

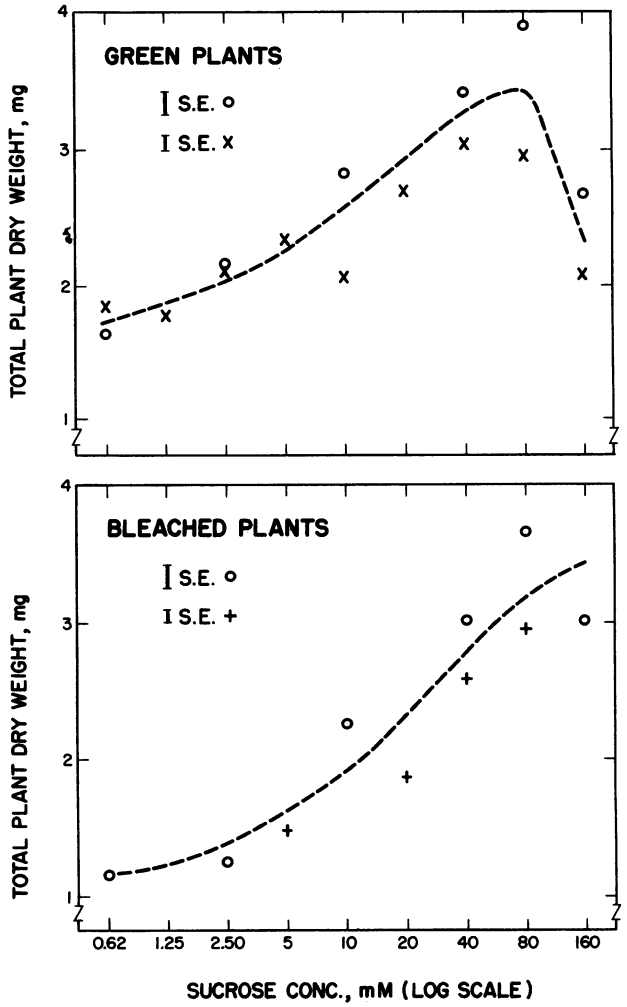

FIG. 4. Effect of sugar concentration on total plant dry weight of *Brassica* grown under an 8-h daylength at 25°C for 12 d. Lines hand drawn through points for two replicate experiments (O, +). Upper, green plants, mean value for −Su control = 1.6 mg, and lower, bleached plants, mean value for −Su control = 1.1 mg. Each point is a mean from 20 to 30 plants.

cell division were controlled by carbohydrates (18), competition between leaf primordia and apical meristematic cells would be reduced by factors increasing the carbohydrate status of the plant, such as increasing light intensities, daylengths, or sugar feeding (Figs. 1–3). Competition would be increased by environmental factors reducing photosynthesis, such as lack of CO_2 (Table IV) or prevention of Chl accumulation (Fig. 3).

Bernier *et al.* (2) have suggested a model for multifactorial control of photoperiodic responses in which both photomorphogenetic factors as well as an energy supply from photosynthates are necessary for the formation of flower primordia. In the experiments reported here the carbohydrate status of the plant was an important controlling factor. Under natural growing conditions, LD plants initiate flowers soon after passing through the critical daylength. As long photoperiods are associated with an increased energy supply from a longer duration of photosynthesis, a high carbohydrate requirement for floral initiation would have the selective advantage of ensuring a high carbohydrate supply for the heavy energy demands of later seed formation.

Table II. *Effect of Sucrose Concentration on Final Leaf Number of Green Plants Grown in an 8-Hour Daylength*

±SE from 15 to 30 replicate plants per treatment.

Sucrose Concn.	Flower Initiation, 14 d old	
	1/8 Hoagland	1/4 Hoagland
mM	%	
0	0	0
40	70	
80	82	64
120		73
	Final Leaf No., 35 d old	
0	5.9 ± 0.22	6.8 ± 0.13[a]
40	5.6 ± 0.18	
80	5.2 ± 0.28	6.0 ± 0.16
120		5.7 ± 0.15[a]

[a] Significantly different final leaf numbers at $< P = 0.01$ level in *t* test.

Table III. *Effect of Osmotic Potential of Medium, Using Different Osmotica, on Flower Initiation of Brassica Grown for 12 Days at 25°C under 8-Hour Daylengths*

Figures are means from 30 plants per treatment.

	Medium							
	Water	Sucrose	Mannitol		NaCl		PEG 400	
Osmotic potential, kPa	−30	−310	−270	−500	−210	−510	−290	−400
Concn., mM		80	80	160	43	87	7% w/v	13% w/v
% Flower initiation	0	79	2.6	3.2	0	0	0	Dead
Mean No. flower buds	0	2.3[a]	0.03[a]	0.03[a]	0	0	0	Dead

[a] SE = ±0.15 from analysis of variance.

Table IV. *Effect of CO_2 Removal on Floral Induction in Brassica*

Plants grown under an 8-h daylength for 12 d with a single supplementary light period of 16 h when 4 d old.

CO_2 Supply		Experiment			$\bar{x}$	SE
Main light period	Supplementary light period	1	2	3		
		% flowering				
Air	Air	93	58	56	69	±14.8
Air	0	17	14	2	11	±5.6[b]
0[a]	Air	46	14	18	26	±12.3[b]
0[a]	0	4	2	3	3	±0.7[b]
8-H control (air)		0	0	0	0	±0.0[b]

[a] CO_2 level held at 0 only for the 4th d after germination.

[b] Significantly lower flowering than air/air control at $< P = 0.05$ level in paired *t* test.

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