Brassica campestris L.: Floral Induction by One Long Day

Abstract. *A strain of* Brassica campestris *L. responds to a single photoinductive cycle 4 days after sowing. Extending the photoperiod from 8 to 22 or 24 hours, with incandescent light of 538-lux intensity, induced inflorescence in 90 percent of the plants. Inflorescence development was visible on dissection 5 or 6 days after photoinduction. Floral induction increased with duration and intensity of the supplementary light.*

The photoperiodic response of long-day plants has not been studied in detail like that of short-day plants, partly because of the shortage of known long-day plants of such high sensitivity and rapid response as the short-day plants *Xanthium, Pharbitis,* and *Chenopodium.* Only three long-day species sensitive to one photoinductive cycle are reported: *Lolium temulentum* (*1*), *Sinapis alba* (*2*), and *Anagallis arvensis* (*3*). A clonal selection of *Trifolium repens* L. is also sensitive to 1 long-day preceded by short days, but will not flower in response to continuous long days (*4*); its response is therefore that of a short–long day plant rather than that of a long-day plant.

Both *S. alba* and *L. temulentum* become sensitive to 1 long day only when about 4 or 5 weeks old. By this time the plants are large, a decided disadvantage in studies of the spectral dependence of floral induction, because of the small areas of high-intensity radiation usually available from spectrographs or when interference filters are used. *Anagallis arvensis* is sensitive in the seedling stage and the plant is small, but the level of flowering in response to one photoinductive cycle is not very high. When incandescent light of 1600-lux intensity is used to extend an 8-hour day length to a 20-hour photoperiod, only 9 percent of seedlings produce an inflorescence (*3*).

We first noticed heterogeneity in the flowering response of the rape variety arlo (*Brassica campestris* L.) in plants grown under continuous illumination. The strain we report on was designated "1-day strain." Progeny from a cross between two early flowering individuals proved to be sensitive to only one photoinductive cycle. Further selection has since been made for high self-compatibility, and ripe seed is obtained about 8 weeks after sowing under greenhouse conditions, with a 16-hour day length.

In photoperiodic experiments, ten seeds are planted in 2-inch (5-cm) diameter plastic beakers containing the growing medium Terragreen, a crushed-clay aggregate; mixtures of perlite and vermiculite, or vermiculite alone, also are satisfactory. Nutrients are withheld in order to limit plant size. Such plants are identical in flowering response with those watered with Hoagland's solution. Seedlings emerge in 1 to 2 days, and the cotyledons open and begin to expand on the 2nd or 3rd day. Under continuous illumination, well-developed flower buds are visible under the dissecting microscope as early as 7 days after sowing, and the final leaf number is usually four. Under short-day conditions (8 hours) plants reach a similar stage of development in about 15 days and the final leaf number is about eight.

Seedling sensitivity to long days was tested by growing plants under 8-, 10-, or 12-hour days (26,900-lux white fluorescent plus 538-lux incandescent) for 3, 4, or 5 days from sowing, and by giving zero, one, two, or four cycles of long days of continuous illumination with a supplementary period of incandescent light (538 lux). Plants were dissected 7 days after commencement of the long-day treatment.

In the 10- and 12-hour-day series, all treatments including 1 or more long days resulted in more than 90 percent of the plants having flower buds at a similar stage of development. The 8- and 10-hour-day controls were all vegetative, but the 12-hour-day plants had well-developed buds. In the 8-hour-day series, 1 long day given as early as 3 days after sowing induced buds in more than 80 percent of the plants. When plants were given 1 long day 4 or 5 days after sowing, the percentage of buds was between 90 and 100 and the buds were more developed.

The response of 4-day-old plants to increasing photoperiod during one photocycle was found by applying supplementary incandescent light of 538-lux intensity at the end of the main light period to provide a series of photoperiods from 8 to 24 hours. Plants were returned to an 8-hour day and dissected 8 days after supplementary light treatment. Both the percentage of floral initiation (Fig. 1) and the stage of bud development were advanced when the photoperiod was lengthened from 14 to 24 hours, in two separate experiments.

The flowering response to 1 long day depends on both intensity and duration of the supplementary light. Plants were grown under 8-hour-day condi-

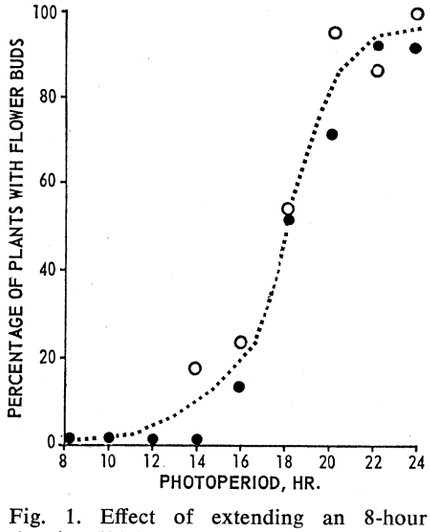

Fig. 1. Effect of extending an 8-hour day by 538-lux incandescent illumination during one photocycle 4 days after sowing. The plants were dissected 8 days after long-day treatments; points, means of 15 plants (solid circles) and 20 plants (open circles) from two replicate experiments, are joined by a freehand curve.

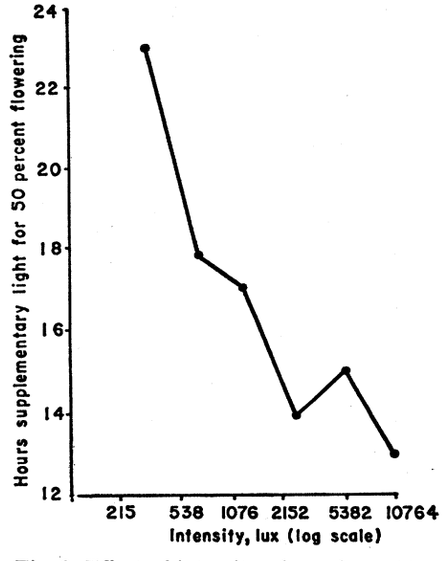

Fig. 2. Effect of intensity of supplementary light on photoperiod necessary for induction of flowers in 50 percent of plants. Basic length of day, 8 hours; plants given one long photocycle when 4 days old and dissected at 10 days.

tions for 4 days and given 1 long day with supplementary light varying in duration from 12 to 24 hours at intensities (incandescent light) varying from 300 to 10,800 lux. The photoperiod needed to induce formation of flower primordia in 50 percent of the plants was estimated by interpolation from a series of response curves similar to Fig. 1; 20 replicate plants were used per treatment. When the intensity was 10,800 lux, a photoperiod of 13 hours was sufficient for 50-percent induction, but, when the supplementary light was reduced to 300 lux, 23 hours were necessary (Fig. 2).

The effect of temperature on sensitivity to 1 long day was tested by growing plants continuously at 15°, 20°, 25° or 30°C. Plants were sensitive to 1 long day at all temperatures. Flower initials were formed most rapidly at 30°C, but the plants were excessively etiolated and growth was poor. Flower initiation was only 1 day slower at 25°C, and, as growth was more satisfactory, this temperature is probably the most useful for further experimentation.

This strain of *B. campestris* is therefore a useful addition to the short list of long-day plants sensitive to 1 long day. There are no special requirements for germination, which is rapid and uniform. The seedlings respond to a single long day as early as 4 days after sowing, and inflorescence development is visible on dissection after another 6 days. Large quantities of seed (about 5000 per plant) can be easily obtained by selfing plants grown under normal greenhouse conditions with a day length of about 16 hours.

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5. We thank the Division of Plant Industry, CSIRO, Canberra, for providing controlled environmental facilities in CERES, L. T. Evans for helpful discussions, and G. Mulligan of this institute for identifying the plant material. Publication 524 of the Plant Research Institute, Canada Department of Agriculture.

13 June 1966

Differentiation in vitro: Effects of Sephadex Fractions of Chick Embryo Extract

Abstract. *Chicken embryo extract has been fractionated into high- and low-molecular-weight components on Sephadex G-25. Media supplemented with the low-molecular-weight fraction (L) support full differentiation in clones of cartilage and of pigmented retina cells from chicken embryos. Growth rates of such cultures in L-supplemented media are greater than in media without embryo extract, and plating efficiencies are higher than in media with or without whole embryo extract. The high-molecular-weight fraction (H) in low concentrations also stimulates growth and plating efficiency, but inhibits the expression of differentiation.*

Chicken embryo extract (EE) has been used to enhance the growth of cells in tissue and organ culture (*1*). High concentrations of EE have marked positive and negative effects on cellular differentiation (*2–4*). Both Ebeling (*3*) and Doljanski (*4*) showed that the amount of pigment formed in plasma clot organ cultures of iris epithelium was inversely proportional to the concentration of EE in the medium. On the other hand, others have reported differentiation of organ explants in media containing 20 to 30 percent EE (*2*). In general, however, non-neoplastic cells in monolayer culture lost overt signs of differentiation in media containing EE unless the media had been conditioned beforehand (see *5, 6*).

In experiments designed to improve methods for growing differentiated cells in monolayer culture or as clones we found that high amounts of EE lowered plating efficiencies with cartilage and pigmented retina. Deletion of EE from cloning media decreased both the growth rates and the plating efficiencies.

In order to separate deleterious factors in EE from those which stimulate growth and promote high plating efficiences, EE was fractionated on Sephadex G-25. Whole chicken embryos (9- to 11-day-old White Leghorn) were mixed with an equal amount (weight/volume) of Hanks' solution (*7*) containing 0.01 to 0.02 mg of phenol red per milliliter, and then minced in an Oster blender at top speed for 40 to 60 seconds. The material was centrifuged in the cold at 34,000 *g* for 30 minutes; the supernatant was then decanted and stirred for 1 hour at 0° to 2°C or was incubated at 0° to 2°C with hyaluronidase (*8, 9*). It was then centrifuged at 100,000 *g* for 85 minutes. The pellet and the lipid layer (pellicle) were discarded, and the remaining clear orange solution was designated whole EE. The EE was either fractionated immediately, or it was kept overnight in the refrigerator and then fractionated (*10*) on a column (2.5 by 80 cm, 400 ml capacity) of Sephadex G-25 (*11*).

Fractionation was carried out at 4° to 6°C at flow rates of 6 to 10 ml/min. Approximately 80 ml of EE was applied to the column and eluted with Hanks' solution (without phenol red). The excluded, high-molecular-weight fraction (H) was that part of the eluate containing hemoglobin as determined visually or by spectrophotometric readings at 410 and 540 mμ. The retarded or low-molecular-weight fraction (L) included the remainder of the fractions up to and including that with the first visible trace of phenol red. The two fractions were sterilized by being forced under pressure through HA or GS Millipore filters, or they were incorporated directly into media which were sterilized as described above after overnight storage at 0° to 2°C. Fractions have been stored up to 2 months in a liquid-nitrogen refrigerator without change in activity. Percentages of L and H were corrected for dilution during fractionation; they refer to the volume of original sample; for example, 5 percent H represents the same concentration of high-molecular-weight components which would be present in a medium containing 5 percent whole EE by volume. Cells were cultured in Ham's medium F10 (*12*) except that twice the amino acid concentrations were used, and supplements of 0.5 to 1.0 percent bovine serum albumin (*13*) and 5 percent fetal calf serum were added.

Plating efficiency, clonal growth rate, and expression of differentiated function were the criteria for the evaluation of media incorporating Sephadex fractions of EE. Cell counts were made with hemocytometers. The criteria for cellular differentiation in chick pigmented retina cells were the degree