Phytochrome control of chlorophyll *a* loss in excised corn leaves requires calcium

Marisa Levi, G. Summer and E. Bellini[1]

Istituto di Scienze Botaniche dell'Università e Centro di Studio del CNR per la Biologia Cellulare e Molecolare delle Piante, via G. Colombo 60, I-20133 Milano (Italy), 9 March 1977

Summary. Phytochrome effect on deferring chlorophyll *a* loss in slices of corn leaves is evident only if incubation is made in the presence of calcium.

Excised green leaves incubated in darkness undergo irreversible degradative processes, such as decrease in protein, nucleic acid and chlorophyll contents[2,3]. Such a syndrome, named senescence, is controlled by light[4-6], hormones (kinetins[2,4] and gibberellins[7-9]) and calcium[10]. The response to light is dependent on photosynthesis in bean leaves[4], while in *Marchantia* a phytochrome reaction seems to be implicated[6]. In recent years some results have suggested that calcium is somehow involved in various phytochrome reactions in vitro (as membrane-binding[11] and dark reversion[12]) and in vivo (Tanada effect in mung bean root tips[13], flowering of *Lemna perpusilla*[14], promotion of anthocyanin synthesis in red cabbage seedlings[15]).

Table 1. Chl a and chl b content (μg/mg fresh weight) and chl a/chl b ratio in slices incubated for 4 days at 25 °C in distilled water or $CaCl_2$ 10^{-3} M

Light treatment	Incubation medium	chl a	chl b	a/b ratio
Darkness	Water	0.524 ± 0.018	0.203 ± 0.006	2.57 ± 0.03
	Calcium	0.687 ± 0.017	0.237 ± 0.009	2.91 ± 0.13
Red	Water	0.571 ± 0.017	0.228 ± 0.006	2.50 ± 0.01
	Calcium	0.742 ± 0.030	0.266 ± 0.014	2.79 ± 0.04
Far red	Water	0.572 ± 0.011	0.216 ± 0.010	2.66 ± 0.08
	Calcium	0.652 ± 0.016	0.237 ± 0.012	2.76 ± 0.08

Red, 2 (7 min FR plus 7 min R) cycles; far red, 2 (7 min red plus 7 min FR) cycles on the 2nd and 3rd day of incubation. The mean of 4 replicates ± SE is presented.

Table 2. Total calcium and phytochrome effects and calcium-phytochrome interactions on chl a

Number of experiment and material	Calcium concentration	Irradiation number	Ca	Pfr	Interaction
1 Discs	10^{-4}	5	None	+**	-
2 Discs	10^{-3}	2	None	None	None
3 Slices	10^{-3}	2	+**	+*	None
4 Slices	10^{-3}	2	+**	+*	+*
5 Slices	10^{-3}	5	+**	+**	+**
6 Slices	10^{-3}	5	+*	+	None

Data of 6 different experiments are elaborated by 2×2 factorial analysis technique. Ca, Calcium effect in a mixed population with active or inactive phytochrome; Pfr, phytochrome effect in a mixed population with or without exogenous calcium added; 'interaction' indicates the combined effect of calcium and phytochrome together. We signify there is no effect (none) when $F < 1$; a positive (+) or negative (-) effect when $F > 1$. * F-value over the 95%; ** F-value over the 99% significance level.

The aim of the present investigation is to test the possibility of a phytochrome effect on the decrease of chlorophyll in excised leaves of *Zea mays* and to ascertain whether an interaction exists between phytochrome and calcium in controlling this phenomenon.

Zea mays 'Dekalb XL 140 A' Fl hybrid was used. Seeds were germinated and grown in sawdust at 25 °C under continuous white fluorescent light. From the first leaf of 7 days old seedlings, 3 discs of 10 mm diameter were punched with a corkborer. In other experiments slices 0.3–0.5 mm thick were cut with a razor blade. Discs or slices were immediately floated on distilled water or 10^{-3} M $CaCl_2$, randomized and transferred in plastic Petri dishes on filter paper with 10 ml distilled water of 10^{-3} M $CaCl_2$ (10 discs per dish, or slices for a total weight of 80–300 mg). The dishes were incubated for 4 days at 25 °C in darkness or in darkness interrupted by different programs of light. At the end of the treatment, chl a and b content was determined by Arnon's method[16] after homogenization and extraction in 80% aceton.

Preliminary experiments showed that the decrease of total chlorophyll is close to linear throughout 6 days in white light, while in the dark the loss rate is sharply enhanced after the second day of incubation. For this reason, in the following experiments we decided to begin the irradiation program 48 h after cutting. The 2-days lag-phase is similar to that found in bean[4] and in *Rumex*[9]. The subsequent drop in chlorophyll content is more marked for chl a, as in *Marchantia*[6] and in *Xanthium*[17], while in Rumex an increase in chl a/chl b ratio was found[9]. The daily change of water in Petri dishes had no significant effect. In agreement with a previous report[10], the presence of $CaCl_2$ in the incubation medium markedly decreased the rate of chlorophyll degradation. In our conditions, the minimal effective concentration of $CaCl_2$

1 Present address: Istituto ed Orto Botanico dell'Università, via Archirafi 38, I-90123 Palermo, Italy.
2 D. J. Osborne, Pl. Physiol. *37*, 595 (1962).
3 H. Thomas and J. L. Stoddart, Pl. Physiol. *56*, 438 (1975).
4 J. J. Goldthwaite and W. M. Laetsch, Pl. Physiol. *42*, 1757 (1967).
5 A. Haber, P. J. Thompson, P. L. Wabne and L. I. Triplett, Pl. Physiol. *44*, 1619 (1969).
6 J. De Greef, W. L. Butler, T. F. Roth and H. Fredericq, Pl. Physiol. *48*, 407 (1971).
7 R. A. Fletcher and D. J. Osborne, Can. J. Bot. *44*, 739 (1966).
8 L. Beevers and F. S. Guernsey, Nature *214*, 941 (1967).
9 J. J. Goldthwaite and W. M. Laetsch, Pl. Physiol. *43*, 1855 (1968).
10 B. W. Poovaiah and A. C. Leopold, Pl. Physiol. *52*, 236 (1973).
11 D. Marmè, J. Boisard and W. R. Briggs, Proc. Nat. Acad. Sci. USA *70*, 3861 (1973).
12 M. Negbi, D. W. Hopkins and W. R. Briggs, Pl. Physiol. *56*, 157 (1975).
13 T. Tanada, Pl. Physiol. *43*, 2070 (1968).
14 R. Halaban and W. S. Hillmann, Pl. Physiol. *48*, 760 (1971).
15 T. A. Hathout Bassim and R. C. Pecket, Phytochemistry *14*, 731 (1975).
16 D. I. Arnon, Pl. Physiol. *24*, 1 (1949).
17 D. W. Newman, B. W. Rewell and K. Byrd, Pl. Physiol. *51*, 229 (1973).

was 10^{-3} M. No significant difference was found whether the discs were incubated or not in the presence of filter paper.

A 2×2 factorial experiment was then set out, in which the factors calcium and state of phytochrome were examined at 2 levels: absence or presence of calcium; active or inactive phytochrome. During the 4 days of the experiments, incubation in darkness was interrupted 2 or 5 times by irradiation cycles consisting of 7 min far red light immediately followed by 7 min red light or vice versa. In the first case (FR–R), phytochrome is assumed to remain in the active form for a longer time in the subsequent dark period; in the second case (R–FR), the inactive form is prevalent. A further control in continuous darkness with or without calcium was arranged.

Typical data from one experiment are reported in table 1: a protective effect of active phytochrome and of calcium on chl a is observed; an increase in chl a/chl b ratio occurs, as calcium by itself is more effective on chl a: this could be ascribed to the higher stability of chl b molecule, but data on the effect exerted by darkness on the rates of chl a and b degradation are rather contradictory[18].

The results of statistical analysis for all experiments are summarized in table 2 for chl a content. Calcium has no significant effect in discs, but prevents chl a loss in slices; this fact can be attributed to the greater length of the cut edge per unit fresh weight in slices than in discs[19]. Phytochrome has a positive action in deferring the loss of chl a and b in slices. Significant positive interaction between calcium and phytochrome was found in 2 experiments. To get the maximum of information we may separate the effects of the single factors by the analysis of variance between 2 groups: relative results for chl a are summarized in table 3. No significant difference exists for the calcium effect in the different light treatments, while phytochrome is effective in preventing the loss of chlorophyll a only in the slices and in the presence of calcium. In summary, calcium and phytochrome interact in a statistically significant way in delaying the loss of chlorophyll a. This finding can explain the failure reported by other authors[4], in the absence of calcium, to demonstrate a phytochrome effect on senescence.

Table 3. Effect of calcium and phytochrome on chl a content in different experimental conditions

Number of experiment and material	Calcium concentration	Irradiation number	Calcium effect			Pfr effect	
			D	R	FR	Water	Calcium
1 Discs	10^{-4}	5	None	—	None	+*	+
2 Discs	10^{-3}	2	None	+	None	None	None
3 Slices	10^{-3}	2	None	+	+**	+	+
4 Slices	10^{-3}	2	+**	+**	+**	None	+*
5 Slices	10^{-3}	5	+**	+**	+**	None	+**
6 Slices	10^{-3}	5	+	+	+	+	+

Actual data were elaborated with the analysis of the variance between 2 groups. For example the calcium effect in darkness was analyzed by comparing each of the groups incubated in continuous darkness with or without calcium. In the same way, phytochrome effect in absence of calcium was obtained by compairing samples in distilled water irradiated with (FR + R) with those irradiated with (R + FR). In experiments 3 and 6 the F-values were 2.08 and 1.10 without calcium and 4.75 and 3.58 with calcium respectively ($F_{0,05} = 5.99$). See table 2 for other explanations. D, darkness.

18 G. A. Lipskaia and L. A. Zelenaya, Fiziologya Rast. *22*, 277 (1975).

19 R. C. Smith and E. Epstein, Pl. Physiol. *39*, 338 (1964).

Migrant selection in a natural population of *Drosophila*

A. Fontdevila, W. T. Starmer, W. B. Heed and J. S. Russel[1]

Departamento de Genética, Facultad de Ciencias, Universidad de Santiago, Santiago de Compostela (Spain), and Department of Ecology and Evolutionary Biology, The University of Arizona, Tucson (Arizona 85721, USA), 11 November 1976

Summary. Migrant flies of *Drosophila nigrospiracula*, a desert species, showed a higher rate of mating than non migrant flies. Increase of mating ability may be favoured by low migration rate, but its causes are not yet well understood. This is the first time that migrant selection is documented from nature.

It has been argued repeatedly that sexual selection plays an important role in evolution and many studies[2–4] have shown that in *Drosophila* sexual selection may be frequency dependent. Although mating behavior of *Drosophila* has been extensively studied under laboratory conditions, very little is known about this behavior in natural populations. The main reason is that it is very difficult to observe a mating actually taking place under natural conditions. In the present work we have tried to learn something on the mating behavior of a desert species of *Drosophila* (*D. nigrospiracula*) under its natural habitat. This species provides the unique opportunity to observe and collect in its natural habitat a sufficient number of mating pairs to make up a workable sample. Since migration is an important factor in the evolutionary history of any species and may play an important adaptive role in desert *Drosophila* species[5], we have attempted to investigate the mating behavior of migrants. Our results suggest that under certain conditions mating may not be at random and migrants may be favoured when rare.

D. nigrospiracula inhabits the Sonoran Desert, which extends through Southern Arizona (USA), Baja California and Sonora (Mexico). Its biology is very well understood[6]. For example it is known to be an oligophagous species feeding on the necrotic tissues of several cacti but mainly on the Saguaro cactus (*Carnegia gigantea*), in the region of Tucson, Arizona, where this study was done.

1 We gratefully acknowledge the field assistance of Robert Mangan, Margaret Jefferson, Don Vacek and Dorthe Jurgenson.

2 C. Petit, Bull. Soc. Zool. Fr. *93*, 187 (1968).

3 L. Ehrman, Am. Nat. *101*, 415 (1967).

4 L. D. Spiess and E. B. Spiess, Am. Nat. *103*, 155 (1969).

5 J. S. Johnston and W. B. Heed, Am. Nat. *109*, 207 (1972).

6 D. P. Fellows and W. B. Heed, Ecology *53*, 850 (1972).