

Phytochrome Controls Achene Germination in *Bidens pilosa* L. (Asteraceae) by Very Low Fluence Response

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ABSTRACT

Achene without ornament of the tegument were light insensitive with germination under all tested light conditions. Achene with verrucose ornament of the tegument presented low germination under darkness and high germination under light conditions. By pre-incubation at 36°C for remotion of pre-existing Pfr and by comparison of results of counting of dark germinating achenes at the end of experiment and daily under dim green safe light ($0.001 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ nm}^{-1}$) we concluded that germination was controlled by phytochrome through very low fluence response.

Key words: *Bidens pilosa*, phytochrome, VLFR, achene germination

INTRODUCTION

Bidens pilosa L. commonly named as picão-preto is responsible for decrease in the yield of several crops (Klingman & Ashton, 1975). Valio et al. (1972) described that light had no effect on achenes germination. Amaral and Takaki (1998) proposed dimorphism was present in *B. pilosa* and that achenes without ornament of the tegument (formerly named as long achenes) had no phytochrome controlling the germination process. However, in achenes with verrucose ornament (formerly named as short achenes) phytochrome controls the germination. Forsyth and Brown (1982) working with *B. pilosa* observed that short achenes presented dormancy and while long ones presented both no dormancy and light sensitivity. Phytochrome controls germination by three distinct mechanisms: very low fluence response (VLFR) characterized by high sensitivity of seeds due to the several environmental factors; low fluence response (LFR) with characteristical photoreversibility and high irradiance response (HIR) where white light can both promote or

inhibit germination depending on the fluences (Mancinelli, 1994). One possibility is that achenes without ornament of tegument with no light sensitivity have phytochrome working at VLFR and controlling the germination process.

In the present work we propose the phytochrome control in both achenes with an without tegument ornament of *B. pilosa* through the VLFR.

MATERIAL AND METHODS

Achenes of *B. pilosa* used in the present work were harvested at the Experimental Garden of University campus and stored in sealed jar at room temperature. The achenes were separated by morphological characteristics with and without verrucose ornament of tegument (Amaral & Takaki, 1998). The germination tests were carried out using 30 achenes on two layers of water moistened filter paper in each of four 90mm Petri dishes (Amaral & Takaki, 1993). Figure 1 shows the spectra of light sources used for incubation and of dim green safe light used for counting of dark

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germinated achenes. Red light (R with $1.085 \mu\text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1}$) was obtained with the aid of one layer or red plexiglass under white fluorescent light. Far red light (FR with $5.358 \mu\text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1}$) was obtained with one layer each of red and blue plexiglass under incandescent bulb, white light (W with $8.93 \mu\text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1}$) with day-light fluorescent lamp and green light ($0.001 \mu\text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1}$) with three layers of green cinemoid and one layer of green plexiglass on one day-light fluorescent lamp. The light spectra were obtained with the aid of a LI-1800 (LI-COR, U.S.A) spectroradiometer. The photoequilibria of phytochrome of used light sources were calculated according to Mancinelli (1994) using the following equation: $\phi = 0.87 / (1 + (0.295/\zeta))$, where, ϕ = photo-equilibrium of the phytochrome and ζ = irradiance at 655-665nm/irradiance at 725-735nm. The analysis of variance of germination results were according to Snedecor (1962) and least significant differences determined at 5% level of probability.

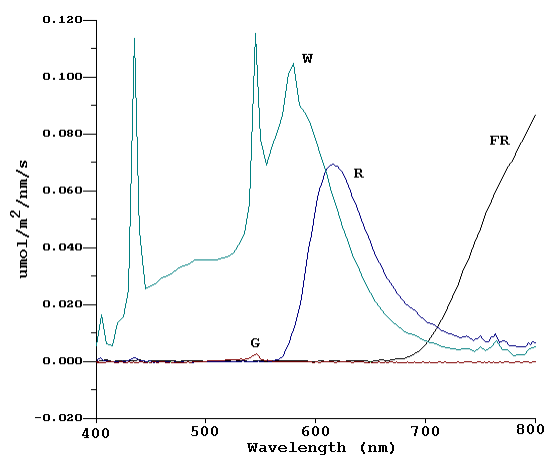


Figure 1 - Light spectra used in the present work. W=white light; R=red light; FR=far-red light and G=green safe light.

RESULTS AND DISCUSSION

Achenes of *B. pilosa* without ornaments presented no light sensitivity and achenes with ornament in the tegument presented low germination under dark and high germination under W as reported by Amaral and Takaki (1998); however, FR was not efficient to inhibit achene germination of this batch of achenes (Figure 2). Fenner (1980) observed that the light requirement is dependent on the conditions of achenes maturation and drying, indicating that because shade light induced

light requirement, phytochrome was present and control germination.

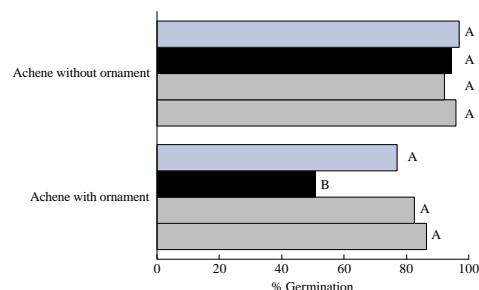


Figure 2 - Germination of achenes of *Bidens pilosa* under different light qualities at 25°C. ■ = White light; ■ = darkness; ■ = red light and ■ = far-red light. The same letter on the figure indicates that there is no significant differences between treatment in the same morphological achene types. The results indicate that achenes without ornaments has no light sensitivity and achenes with ornament germinate under light conditions and low germination in darkness (experiment carried out with achenes with 5 months storage).

One possibility could be that the level of pre-existing Pfr was high enough to promote germination under darkness. To test this possibility, achenes were incubated at 36°C during 36 hours in darkness (Takaki & Gama, 1998). Decrease in the germination percentage under dark indicated that Pfr was removed below the threshold needed for the induction of germination, however, FR still inefficient to inhibit germination (table 1). Because the source of FR maintains 5% of Pfr (Mancinelli, 1994), another possibility could be that the germination of achenes of *B. pilosa* was controlled by phytochrome through the VLFR, where 5% of Pfr was enough to promote complete germination. Because the processes controlled by phytochrome under VLFR were very sensitive, dim green safe light currently used to monitor germinated seeds had light fluence enough to saturate phytochrome controlled germination (Kendrick & Cone, 1985). To prove if the source of green light could promote germination of achene in *B. pilosa*.

Table 1 - Effect of pre-incubation at 25°C and 36°C during 48 hours on the germination under different light qualities in achenes of *B. pilosa*. W= white light; D=darkness; R=red light and FR=far-red light. The same letter are not significant at 5% probability level.

	Temperature	W	D	R	FR
Achenes without ornament	25°C	96.37A	88.33AB	80.83B	83.33B
	36°C	95.37A	32.50C	73.33B	96.37A
Achene with ornament	25°C	37.50A	37.53A	40.00A	39.10A
	36°C	36.60AB	8.33C	48.33A	21.67B

The achenes without ornament on the tegument incubated at 25°C presented germination under all light conditions when monitored daily under green light. However, when counted only at the end of the experiment, low percentage germination were obtained under darkness and FR irradiation (Figure 3), indicating the phytochrome control of achene germination by VLFR. The data obtained by Valio et al. (1972) where light had no effect, indicating no phytochrome participation, was due to the fact that the VLFR was described by the first time in 1981 by Mandoli and Briggs in oat seedlings. Before that date, there are no reports about phytochrome controlling germination of "photo-insensitive" seeds. Our results indicated that for characterization of phytochrome control, all seed germination studies at least four distinct experiments must be carried out: i. incubation at a constant temperature; ii. incubation at high temperature in darkness to remove possible pre-existing; iii. Pfr counting of dark germinating seeds periodically under dim green safe light; iv. counting of dark germinating seeds only at the end of the experiment.

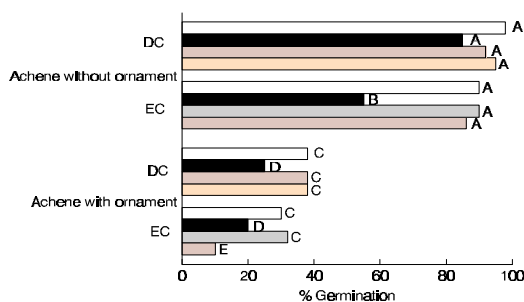


Figure 3 - Effect of daily counting of dark germinating achenes under dim green safe light (DC) and counting only at the end of the experiments (EC). Incubation conditions: = white light; ■ = dark; ≡ = red light; ||| = far red light (experiment carried out with achenes with 1 month storage).

RESUMO

Aquênios sem ornamento do tegumento são insensíveis à luz com ocorrência de germinação sob todas as condições de luz testadas. Aquênios com ornamento verrucoso do tegumento apresentou baixa germinação sob escuro e alta germinação sob luz. A pré-incubação a 36°C para a remoção de Fve pré-existente e pela comparação dos resultados de contagem no final do experimento de aquênios que germinam no escuro e diárias sob luz verde de segurança ($0.001\mu\text{mol m}^{-2}\text{s}^{-1}\text{nm}^{-1}$) concluímos que a germinação de *Bidens pilosa* é controlada pelo fitocromo através da resposta de fluência baixa.

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