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BRIEF COMMUNICATION

Effects of irradiance on photosynthesis and activity of protease inhibitors in *Amaranthus hypochondriacus*

Y. NAGAMATSU-LÓPEZ*, A. BLANCO-LABRA**, J. DÉLANO-FRIER** and E. PIMIENTA-BARRIOS*[1]

*Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Km 15.5 Carretera a Nogales, Las Agujas, 45110 Zapopan, Jalisco, México**
*Centro de Investigaciones y Estudios Avanzados del Instituto Politécnico Nacional, Unidad Irapuato, Km 9.6 Libramiento Carretera Irapuato-León, 36500 Irapuato, Guanajuato, México***

Abstract

Amaranthus hypochondriacus plants were grown under three photosynthetic photon flux densities (PPFD). Mature plants grown at full sunlight (38.8 mol m^{-2} d^{-1}) had higher maximum net photosynthetic rate (P_N) and significantly higher leaf trypsin inhibitor activity than plants that developed under lower PPFD (19.4 and 12.8 mol m^{-2} d^{-1}). In contrast, seeds collected from plants fully exposed to sunlight showed the lowest activity of trypsin inhibitor, higher rate of germination and susceptibility to infection by *Aspergillus niger*.

Additional key words: net photosynthetic rate, seed germination, trypsin inhibitor.

Protease inhibitor proteins (PIs) have a role in plant defense (Walker-Simmons and Ryan 1977, Johnson *et al.* 1989, Lorito *et al.* 1994, Koiwa *et al.* 1997). A typical response to mechanical wounding or insect attack is the induced accumulation of trypsin- and chymotrypsin-PIs in tomato and potato plants. In these plants, PIs accumulate both locally in the wounded leaf, and systemically in the peripheral, unwounded leaves (Ryan 1990). Because little information is available on the abiotic factors, particularly irradiance, that affect PIs synthesis in plants, we studied photosynthesis and the accumulation of PIs in both seeds and leaves of *Amaranthus hypochondriacus* Mill. plants exposed to different irradiances. It was hypothesized that the magnitude of PIs activity in both vegetative and reproductive tissues of *A. hypochondriacus* might be energetically dependent on photosynthesis. Consequently, plants of *A. hypochondriacus* exposed to different levels of irradiances will present variations in PIs activities.

Seeds of *Amaranthus hypochondriacus* Mill. cv. Nutrisol, were collected from one progenitor and sown in 48 plastic containers in February 2000 at the experimental field of the Departamento de Ecología of the Universidad de Guadalajara. Ten days later each seedling was assigned to one of three irradiances replicated 16 times, in a completely randomized design. One group was covered with a plastic net that attenuated 67 % of the incident sunlight (equivalent to a total irradiance of 12.8 mol m^{-2} d^{-1}); in the second group incident sunlight was attenuated 50 % (19.4 mol m^{-2} d^{-1}) with a plastic net too, and the third group was exposed to full sunlight (38.8 mol m^{-2} d^{-1}). Eleven weeks after emergence, when the plants were fully mature, net photosynthetic rates (P_N) were measured on twelve plants per treatment, on the 8th June 2000. Measurements were realized using a *LI-6200* portable photosynthesis system (*Li-Cor*, Lincoln, USA) equipped with a 250 cm^3 *Li-Cor* leaf chamber. Photosynthetic photon flux densities (PPFD; 400 to 700 nm) were measured simultaneously using a *Li-Cor 190S* quantum sensor. On the same date, 10 leaf sections (1.54 cm^2) of each of 12 plants per treatment were cut from leaves using a cork-borer, frozen in liquid nitrogen, and then grounded into a fine powder. The ground material was suspended in 0.6 cm^3 of

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Abbreviations: PIs - protease inhibitor proteins; P_N - net photosynthetic rate; PPFD - photosynthetic photon flux density.
[1]Corresponding author present address: Cerrada de la Maestranza 1098, Residencial Guadalupe Jardín, 45030 Zapopan, Jalisco, México; phone and fax: (+52) 333 777 1156, e-mail: epimient@cucba.udg.mx

deionized-distilled water and subsequently homogenized for 5 min. The mixture was centrifuged at 17 000 *g* for 15 min at 4 °C. The supernatant was re-centrifuged at the same speed for 1 min to eliminate the remaining plant debris. The resulting extract was assayed for protein content (Bradford 1976). Inhibitory activity against bovine trypsin was determined as described by Schwertz and Takenaka (1955) and expressed as trypsin inhibitor units (TIU) per mg of protein. Similarly, protein content and trypsin inhibitor activity in seeds were determined using aqueous extracts prepared from 0.05 g of ground seed flour. To test germination efficiency, 6 replicates of 20 seeds were placed on two layers of *Whatman No. 1* filter paper at 20 °C under constant irradiance (355 μmol $m^{-2}s^{-1}$) in a 9-cm Petri dish, and soaked with 8 cm^3 of distilled water. Germination was scored when the radical length surpassed 2 mm, and expressed as the percentage of germinated seeds within 10, 20 and 30 d of imbibition treatment. Data were subjected to *ANOVA* and when significant, means were separated by a least significant difference (LSD) test.

Mature *A. hypochandriacus* plants grown at full sunlight had the highest maximal P_N and highest trypsin inhibitor activity (Table 1). The influence that PPFD exerts on the basal levels of trypsin inhibitor activity in leaves and seeds of *A. hypochondriacus* plants indicates that the activity of this inhibitor is not only related to a response to wounding or insect attack, but may also be influenced by environmental factors such as the irradiance. Since protease inhibitors are associated with plant defense responses (Koiwa *et al.* 1997), a light-regulated accumulation of trypsin inhibitors in the leaves of *A. hypochondriacus* could have been part of a constitutive defense mechanism that requires high PPFD to be efficiently expressed (Ryan 1990). It is therefore suggested that PPFD must be considered in future experiments with protease inhibitors under controlled conditions, in which irradiance varies from 7 to 14 mol m^{-2} d^{-1} (Ryan 1990), corresponding to 18 - 35 % of the daily average values of full sunlight at the study site.

On the other hand, trypsin inhibitor activity was lowest in those seeds produced by plants grown under the highest PPFD, but they showed the highest rates of germination during the first 20 d after water imbibition (Table 1). An unexpected observation was that 80 % of seeds of *A. hypochondriacus* plants grown under the highest PPFD were infected with the fungi *Aspergillus niger*. In contrast, seeds of plants exposed to 50 and 33 % of the sunlight showed 50 % and 10 % of infection, respectively. Indeed, both trypsin and chymotrypsin inhibitors had shown to be effective against fungal infection (Lorito *et al.* 1994, Mosolov *et al.* 1976). Further work would be required in order to prove the relationship between trypsin inhibitors in seeds of *A. hypochondriacus* and susceptibility to fungi.

Table 1. Maximum net photosynthetic rate (P_N), trypsin inhibitor activity in leaves and seeds, and seed germination rates for *Amaranthus hypochondriacus* grown under different photosynthetic photon flux densities (PPFD). Data are means (n = 12 for maximal P_N and trypsin inhibitory activity and 6 for germination rate). Means followed by the same letter within a column are not statistically different at $P < 0.05$ by LSD's multiple test.

PPFD [mol m^{-2} d^{-1}]	P_N [μmol m^{-2} s^{-1}]	Trypsin inhibitor activity [TIU mg^{-1}]		Germination rate [%]		
		leaf	seed	10 d	20 d	30 d
38.8	16.6	45a	17b	65a	82a	87a
19.4	11.7	26b	22ab	52ab	70ab	84a
12.8	4.8	20c	27a	24b	40b	82a

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