Irradiance stress responses of gas exchange and antioxidant enzyme contents in pariparoba [*Pothomorphe umbellata* (L.) Miq.] plants

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Abstract

We evaluated the growth and development of the medicinal species *Pothomorphe umbellata* (L.) Miq. under different shade levels (full sun and 30, 50, and 70 % shade, marked as I_{100}, I_{70}, I_{50}, and I_{30}, respectively) and their effects on gas exchange and activities of antioxidant enzymes. Photosynthetically active radiation varied from 1 254 μmol m^{-2} s^{-1} at I_{100} to 285 μmol m^{-2} s^{-1} at I_{30}. Stomatal conductance, net photosynthetic rate, and relative chlorophyll (Chl) content were maximal in I_{70} plants. Plants grown under I_{100} produced leaves with lower Chl content and signs of chlorosis and necrosis. These symptoms indicated Chl degradation induced by the generation of reactive oxygen species. Stress related antioxidant enzyme activities (Mn-SOD, Fe-SOD, and Cu/Zn-SOD) were highest in I_{100} plants, whereas catalase activity was the lowest. Hence *P. umbellata* is a shade species (sciophyte), a feature that should be considered in reforestation programs or in field plantings for production of medicinal constituents.

Additional key words: catalase; chlorophyll; oxidative stress; photosynthesis; shade plants; stomatal conductance; superoxide dismutase; transpiration rate.

Introduction

Photon energy is the only source of energy for plants but it can have harmful effects on plants if irradiance is lower or higher than the physiological requirement for plant growth and development (Long *et al.* 1996, Lawlor 2001, Loomis and Connor 2003, Mandal and Sinhá 2004). High leaf irradiance reduces photosynthetic efficiency resulting in photodynamic degradation of the photosynthetic apparatus. Pigment-protein complexes present in photosystem 2 are highly sensitive to photo-damage triggered by the formation of reactive oxygen species (ROS) (Barber and Anderson 1992).

Plants have developed enzymatic and non-enzymatic protection mechanisms against irradiance stress. The non-enzymatic ROS scavenging mechanism includes major redox buffers such as gluthatione, tocopherol, flavonoids, alkaloids, and carotenoids. These antioxidants constitute a protection mechanism that efficiently quenches the triplet state of chlorophyll (Chl) and free radicals formed during high irradiation (Apel and Hirt 2004). Superoxide dismutases (SOD, EC 1.1.15.1) and catalase (CAT, EC 1.11.1.6) are part of the enzymatic antioxidative response system (Asada 1996, Niyogi 1999). These enzymes are in different cellular compartments and are controlled by a ROS gene network (Mittler *et al.* 2004). Three types of SOD have been described in plants: mitochondria-located Mn-SOD, plastid-located Fe-SOD, and Cu/Zn-SODs which are divided in two classes, a cytosolic and a plastidic isoform. According to Apel and Hirt (2004), SODs act as the first line defense against ROS, dismutating superoxide-radicals to H_2O_2 (Bowler *et al.* 1994, Kanematsu and Asada 1994), and CAT subsequently detoxifies H_2O_2 (Hertwig *et al.* 1992).

Habitat fragmentation leads to microclimate changes that expose the remaining species, especially shade-adapted plants, to higher irradiance than normal. The Atlantic Forest is the most fragmented biome in Brazil (Tabarelli *et al.* 2005). There is an ongoing effort to propagate native species to recover fragmented areas or

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to develop the native species with commercial value into new crops. *Pothomorphe umbellata* (L.) Miq. [syn. *Heckeria umbellata* (L.) Kunt., *Piper umbellata* L., *Piper hilarianum* Stend.] is a commercially important medicinal species native to the Atlantic Forest that belongs to the Piperaceae family (cf. Marchese *et al*. 2006).

According to Noriega *et al.* (2008), the pharmacologic importance of *P. umbellata* was first described by Pison in 1638 in "De Medicinal Brasiliensis". *P. umbellata* was later included in the first edition of the Brazilian Pharmacopeia as "pariparoba" dried roots used as a medicinal drug. Perrazo *et al.* (2005) have confirmed the anti-inflammatory and analgesic properties of *P. umbellata* leaf extracts using an animal model, which supports its traditional use in the treatment of inflammatory disorders. In addition, 4-nerolidylcatechol, found in leaves and roots of pariparoba, is the active constituent responsible for potent antioxidant activity on the skin and an effective reduction of UV-B-skin photo-aging (Ropke *et al*. 2003, 2005, 2006).

While the therapeutic value of *P. umbellata* was previously reported (Barros *et al.* 1996, Lorenzi and Matos 2002) with isolation of lignans, neolignans, flavonoids, and alkaloids, confirming its traditional uses (Kijjoa *et al.* 1980, Rorig and Von Poser 1997, França 1999), little is known about the species' growth requirements. We evaluated under different shading gas exchange and the activity of antioxidant enzymes of *P. umbellata*.

Materials and methods

In January of 2003, seeds from *P. umbellata* were sown in plastic trays using *Plantmax*®, a commercial peat moss substrate. Two-month-old seedlings were transferred to plastic containers and maintained in the nursery until October, 2003. The experiment was carried out at the experimental area of the Agronomic Sciences College, São Paulo State University, Brazil (22°51'S, 48°26'W, 786-m height).

After the first rain, the plants were transplanted in holes of 60×60×60 cm spaced 0.8 m within the row. Rows were spaced 1 m apart. A total of five plants were cultivated per row. Each row was covered with shade screens varying between 30 and 70 %, and the full sun control (marked as I_{70}, I_{50}, I_{30}, and I_{100}). The experiment design consisted of randomized blocks with four replications.

Canopy gas exchanges of 14.5-month-old plants were measured using a *LI-6200* portable photosynthetic system (*LI-Cor*, Lincoln, NE, USA). Measurements were taken at 10:00 and 11:00 h. Inside of the photosynthetic camera, the air temperature was 25.5±0.8 ºC, with a deficit of steam pressure of 21.4±2.5 kPa, relative humidity of 65.6±8.3 %, and external CO_2 concentration of 331.6±13.4 mol(CO_2) mol^{-1}. The average photosynthetically active radiation (PAR) was 1 254, 836, 581, and 285 μmol m^{-2} s^{-1} for I_{100}, I_{70}, I_{50}, and I_{30}, respectively.

A chlorophyll (Chl) meter (*SPAD-502*, *Minolta Camera*, Osaka, Japan) was used to record Chl content in SPAD values, according to procedure of Yadava (1986). Fully expanded leaves from the top of six randomly selected plants were used for SPAD measurement. Five readings per plant were made.

The content of soluble protein was determined by the Bradford's assay (1976) using bovine serum albumin as the protein standard. Native polyacrylamide gel electrophoresis was carried out at 4 ºC for 90 and 120 min for SOD and CAT, respectively, at 200 V constant-voltage on 12.5 % polyacrylamide gels for SOD and 8 % gels for CAT, using the Laemmli (1970) buffer system without SDS. After incubation, the gels were irradiated until SOD bands became visible. CAT activity was quantified by the visual staining method of Woodbury *et al*. (1971).

The experiment was established in a randomized design with four treatments and four replications. Collected data were submitted to analysis of variance and regression analysis by orthogonal polynomials.

Results and discussion

I_{70} plants had the highest net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and Chl content (Fig. 1). These parameters were lower in I_{100}, I_{50}, and I_{30} plants than in I_{70} plants. Our results corroborate with the biometric data reported by Mattana *et al.* (2006) that show higher leaf biomass for *P. umbellata* cultivated at 30 % shade. The reduction of P_N in I_{100} was due to the Chl content decrease, while lesser P_N in I_{50} and I_{30} plants could be due to the radiation decreases above the plant canopy. Leaf temperature (T_L) showed a trend similar to P_N at I_{50} and I_{30}, with a reduction of 2.1 ºC at I_{30}, if compared to I_{70} plants. In forage species, Wong and Wilson (1980) described that T_L of plants maintained under shade cloth (40 and 60 % of shade) showed a 1−2 ºC decrease in T_L in comparison to plants grown under sunlight at noon. Fay and Knapp (1995) reported that leaf gas exchange in sorghum, soybean, and eastern gama-grass, under leaf short-term shading, at 300–400 μmol m^{-2} s^{-1} PPFD reduced P_N, T_L, and E, and increased intercellular CO_2 partial pressure (C_i).

Leaf chlorosis and necrosis were noticed in *P. umbellata* grown under full sun (Fig. 2). These symptoms may be the result of Chl photodynamic degradation. According to Asada (1999) plants receiving excess photon energy have an increase in the singlet and triplet forms of Chl and singlet oxygen. The depletion of the $NADP^+$ pool induced by energy in excess promotes an increase in the rate of electron flow from the donor side of photosystem 1

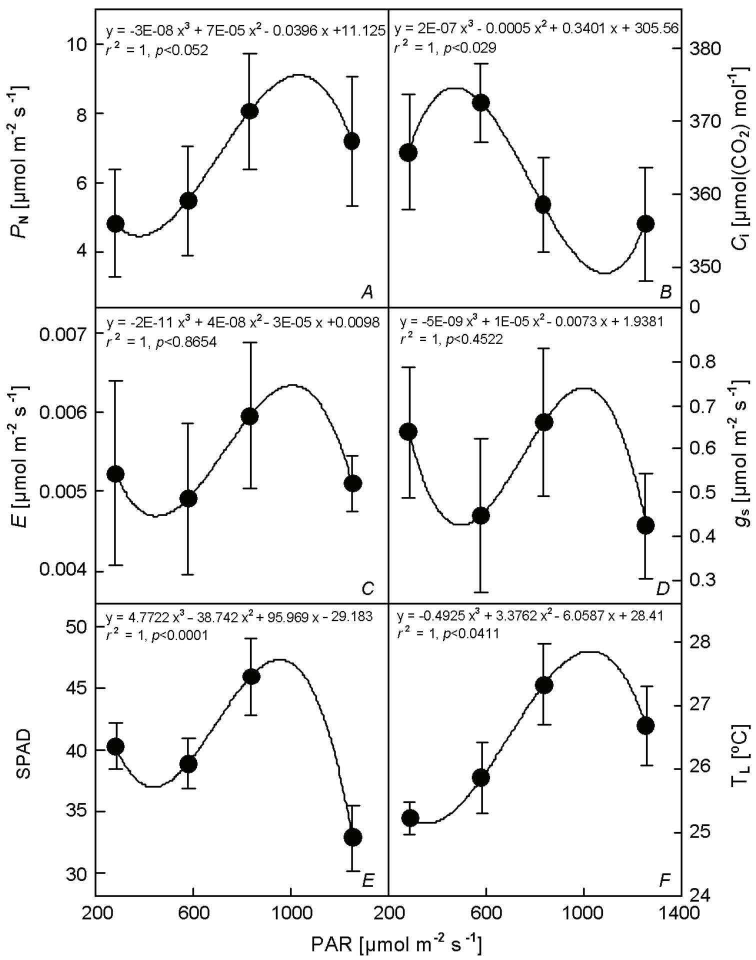

Fig. 1. Effects of photosynthetically active radiation on (*A*) net photosynthetic rate, P_N, (*B*) intercellular CO_2 concentration, C_i, (*C*) transpiration rate, *E*, (*D*) stomatal conductance, g_s, (*E*) chlorophyll content in SPAD units, and (*F*) leaf temperature, T_L of *Pothomorphe umbellata*. Means ± S.D.

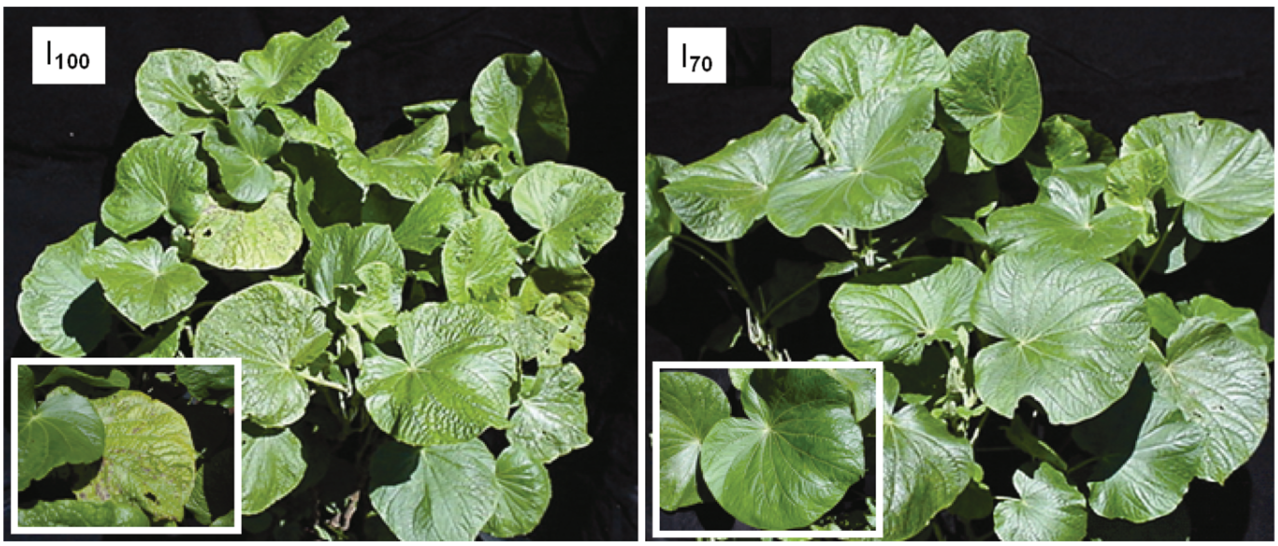

Fig. 2. *Pothomorphe umbellata* plants grown in full sun (I_{100}) and 30 % shade (I_{70}). Signs of chlorosis and necrosis are found in I_{100} plants.

to oxygen with increasing ROS such as superoxide and hydrogen peroxide. Niyogi (1999) reported that enhanced ROS generation may result in lipid peroxidation and massive membrane photodestruction, leading to cell death if ROS are not removed.

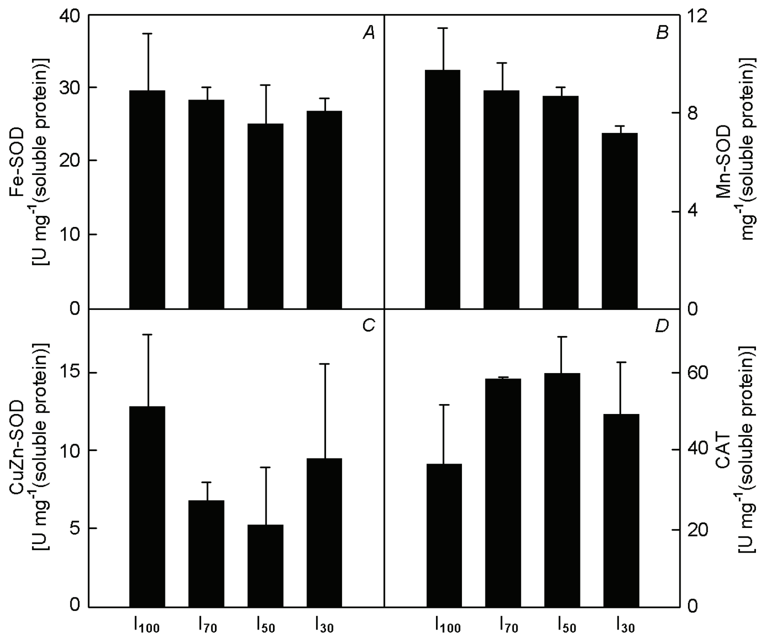

Fig. 3. Changes in activities of superoxide dismutase, SOD (*A* – Fe-SOD, *B* – Mn-SOD, *C* – Cu/Zn SOD) and catalase (CAT) in crude extract from the leaves of *Pothomorphe umbellata* grown under different irradiances. Means ± S.D.

CAT and SOD are part of the enzymatic ROS scavenging mechanisms that plants have evolved to reduce ROS damaging effects. The three SODs, Cu/Zn SOD, Fe-SOD, and MnSOD, involved in converting superoxide to H_2O_2 and oxygen, were at their highest contents in I_{100} plants (Fig. 3). These activities were probably induced by high contents of ROS. Mishra *et al.* (1993) reported similar findings on the gradual increase in SOD activities in irradiated wheat leaf extracts due to increased ROS generation. Logan *et al.* (1998) working with *Cucurbita pepo* and *Vinca major* also verified SOD increase with increasing growth PPFD in both plant species.

Fe-SOD activity (Fig. 3*A*) was higher under the four treatments when compared to the other SODs (Mn and Cu/Zn). Increasing shade did not show any significant effect on activities of SOD isoforms although a decreasing trend was noticed; these results suggest low generation of ROS under shade, insufficient to trigger ROS in signaling genes responsible for activation of the SODs. In contrast to SODs, CAT presented a well-defined increase in activity under shade treatments. Thus, CAT activity was lowest in I_{100} plants, suggesting photolability. Degradation of CAT occurs at high irradiance in other species (Feierabend and Engel 1986, Hertwig *et al.* 1992, Mishra *et al.* 1993, Broetto *et al.* 2002).

Combining the results on *P. umbellata* canopy gas exchange and the activities of SODs and CAT, we conclude that this is an obligate shade species (sciophyte). Thus, to develop *P. umbellata* as a medicinal crop for production of leaf biomass containing 4-nerolidylcatechol to manufacture anti-aging creams, or to include the species in the reforestation programs to recover the Atlantic Forest biome, shade growing conditions must assure optimum *P. umbellata* metabolism.

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