

# Seed vigor, antioxidant metabolism and initial growth characteristics of red rice seedlings under different light intensities

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## ABSTRACT

This work aimed to evaluate the effect that different intensities of light have on the physiological attributes of red rice seeds and seedlings. Before and after emergence, seedlings were exposed to light levels of 35%; 65% and 100% in a greenhouse. We evaluated shoots and roots, in terms of length and dry mass, as well as leaf area and content of chlorophyll (*a*, *b* and total). In leaves and roots, we quantified the activity of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and guaiacol peroxidase (POX). We determined the leaf area ratio ( $F_A$ ), leaf mass ratio ( $F_W$ ), specific leaf area ( $S_A$ ), and shoot/root ratio ( $P_W$ ). At higher light intensities, shoot length decreased, whereas root length, dry mass and number of tillers increased. Leaf area was greatest in seedlings exposed to a 65% light level. The  $F_A$ ,  $F_W$ ,  $S_A$  and  $P_W$  were lowest at a light intensity of 100%. Differences in light intensity had qualitative and quantitative effects on chlorophyll contents. The activity of SOD and CAT was higher at lower light levels, whereas the inverse was true for APX and POX activity. Extremes of light availability alter the activity of antioxidant enzymes, negatively affecting the initial growth characteristics and photosynthetic pigments of red rice seedlings.

**Key words:** *Oryza sativa* L., shading, chlorophyll content, antioxidant enzymes

## Introduction

Irrigated rice is one of Brazil's main annual crops, national production reaching approximately 11,268 thousand tons per year (CONAB 2012). Red rice, a plant that belongs to the same species, is the principal weed of cultivated rice (Villa *et al.* 2006). Red rice is quite robust and resistant to adverse environmental conditions, as well as having a high seed shattering rate, making it hard to control. Its morphological and physiological similarity to cultivated genotypes also renders it difficult to control (Balbinot Junior *et al.* 2003).

The degree to which a weed interferes with the cultivation of a crop varies from species to species and can have a negative effect on the growth and development of the crop due to competition for water, light and nutrients. By photosynthesis, plants absorb radiant energy and fix atmospheric carbon dioxide in carbon compounds for growth and development (Marenco & Lopes 2005). Crop canopy shading reduces the amount of solar radiation reaching some of the leaves of plants and consequently decreases the amount of radiation absorbed by the photosynthesis apparatus. This

tends to reduce the conversion of light energy into chemical energy and can alter the physiological variables of growth, which is influenced in management and adaptive situations.

Superoxide dismutase (SOD), peroxidases and catalases are enzymes involved in the removal of free radicals during the process of seed deterioration (Rosa *et al.* 2005), as well as in seedlings and plants under adverse environmental conditions. Seed vigor consists of a set of physiological processes and involves the ability to reorganize the cell membrane system, as well as hydrolysis, translocation and allocation of seed reserves to the embryonic axis, making it an important parameter for evaluating the quality of the seed. Seed vigor is routinely determined by the emergence of seedlings. Therefore, the determination of the activity of antioxidant metabolism enzymes, used in combination with the evaluation of photosynthetic pigments and the characteristic of early growth and vigor of seeds, provides a better understanding of the effect that the amount of light energy available has on the performance of red rice seeds and seedlings.

There have been few studies on seed vigor, antioxidant metabolism, photosynthetic pigment content and early

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growth characteristics in weeds, and there are no available data on these physiological attributes in red rice seedlings exposed to different levels of light intensity. Such evaluations would be worthwhile, not only because it is known that the response to changes in the level of radiant energy varies widely among species but also because of the negative effect that competition between red rice and cultivated rice has on the productivity of the latter. Therefore, the objective of the study was to analyze the effect that exposure to different levels of light intensity has on the physiological attributes of red rice seeds and seedlings.

## Material and methods

The work was conducted in a chapel-style greenhouse, with a north-south orientation (31°52'S; 52°21'W, at 13 m above sea level) at the Federal University of Pelotas, located in the city of Pelotas, which is in the state of Rio Grande do Sul, Brazil. According to the Köppen climate classification system, the climate of the region is type Cfa, a temperate climate with well distributed rainfall and hot summers. Analyses were carried out at the Laboratory of Seed Physiology of the Graduate Program in Seed Science and Technology of the Federal University of Pelotas.

The physiological status of non-dormant red rice (*Oryza sativa* L.) seeds was determined by evaluation of germination and by a tetrazolium test. The seeds were then incubated, at a depth of three times their length, in polyethylene trays containing planosol, previously adjusted according to soil analysis and based on the Fertilization and Liming Manual for the State of Rio Grande do Sul (CQFS-RS/SC 2004).

The trays were laid on a concrete floor under shading screens (Sombrite) supported by wooden rectangular structures of 1.5 m in height, arranged in such a manner as to isolate the top and sides of the structures, in order to establish different levels of light intensity.

Irrigation was performed manually, with a fine sprinkler, without removing the shading screens and carried out as needed in order to maintain soil moisture in its field capacity. The shading screens were removed 21 days after sowing for the purpose of enabling the evaluation of seedling emergence, as well as the collection of plant material for analysis of chlorophyll content, antioxidant enzyme activity and initial growth.

The seeds and seedlings remained in the greenhouse environment, where they were exposed to one of three levels of light intensity: 35%, 65% or 100%.

The influence that each level of light intensity had on the various physiological attributes of red rice seeds and seedlings was evaluated by measuring the following parameters: *Emergence of seedlings in the greenhouse*: conducted in four subsamples of 50 seeds each. A final count of the number of emerging seedlings was carried out 21 days after sowing, and the results are expressed as the percentage of seedlings emerged.

*Length of the shoots and roots of the seedlings*: evaluated in four subsamples of 10 seedlings each, at the end of the emergence test in a greenhouse, at 21 days after sowing. The length of the shoot was determined by measuring the distance between the insertion of the basal portion of the root to the apex of the shoot, whereas root length was determined by measuring the distance between the apex and the base. The results are expressed in millimeters per seedling ( $\text{mm organ}^{-1}$ ).

*Dry mass of the shoots and roots of the seedlings*: obtained by measuring the mass of four subsamples of 10 seedlings each, at the end of the emergence test in a greenhouse, at 21 days after sowing. The seedlings were sorted into organs, separately packaged in brown paper envelopes and dried in a forced ventilation oven at a temperature of 70°C, for 72 h. The results are expressed in milligrams per seedling ( $\text{mg organ}^{-1}$ ).

*Leaf area of the seedlings*: determined by measuring the leaf area of four subsamples of 10 seedlings each, with an area meter (LI-3100; LI-COR, Lincoln, NE, USA), at the end of the emergence test in a greenhouse, at 21 days after sowing.

*Leaf area ratio, leaf mass ratio, specific leaf area, shoot and root ratio and number of tillers*: determined from the primary data of organs and leaf area dry mass growth. The values for leaf area ratio ( $F_A$ ), leaf mass ratio ( $F_W$ ), specific leaf area ( $S_A$ ) and shoot and root ratio ( $P_W$ ) were estimated using the following equations (Radford 1967):

$$\begin{aligned} F_A &= A_F/W_T \\ F_W &= W_F/W_T \\ S_A &= A_F/W_F \\ P_W &= W_S/W_R \end{aligned}$$

where  $A_F$  is the leaf area,  $W_T$  is the total dry mass given by the sum of the dry matter of the shoot and roots,  $W_F$  is the dry mass of leaves,  $W_S$  is the dry mass of the shoot, and  $W_R$  is the dry root mass. The number of tillers was determined by direct count.

*Chlorophyll a, chlorophyll b and total chlorophyll content in seedlings submitted to the emergence test*: quantified, at the end of the emergence test, in four samples of plant tissue. This fresh plant material was macerated in a mortar and pestle with 10 ml of acetone 80%, in a darkroom under green light. The macerated material was subjected to a simple filtration and acetone was added up to a final volume of 25 ml. The readings were taken in a spectrophotometer with absorbance at 645 nm and 663 nm (Arnon 1949). Chlorophyll content is expressed as mg of chlorophyll  $\text{g}^{-1}$  fresh mass.

*Determination of antioxidant enzyme activity*: carried out in samples of fresh seedling material obtained at the end of the emergence test, macerated in a mortar and pestle with liquid nitrogen, containing 20% polyvinylpyrrolidone and homogenized in 1.8 ml of potassium phosphate buffer 100 mM (pH 7.8) containing 0.1 mM EDTA and 20 mM

ascorbic acid. The extract was centrifuged at  $13.000 \times g$  for 20 min at  $4^{\circ}\text{C}$ , and the supernatant was used in order to measure enzyme activity.

*SOD (EC 1.15.1.1) activity*: evaluated by the capacity to inhibit the photoreduction of nitroblue tetrazolium (NBT) at 560 nm in a reaction medium containing 50 mM potassium phosphate buffer (pH 7.8), 14 mM methionine,  $0.1 \mu\text{M}$  EDTA, 75 NBT and  $2 \mu\text{M}$  riboflavin, according to Giannopolitis & Ries (1977). One unit of SOD activity was defined as the amount of enzyme produced from a 50% inhibition of the photochemical reduction of NBT.

*Ascorbate peroxidase (APX - EC 1.11.1.11) activity*: determined by the methodology proposed by Asada & Nakano (1981) by means of monitoring the oxidation rate of ascorbate for 2 min at 290 nm ( $\epsilon = 2.80 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ ). The reaction medium, consisting of 100 mM (pH 7.0) potassium phosphate buffer, 0.5 mM ascorbic acid, 0.1 mM hydrogen peroxide and enzyme extract, was incubated at  $30^{\circ}\text{C}$ .

*Catalase (CAT - EC 1.11.1.6) activity*: determined as described by Azevedo *et al.* (1998). The activity was monitored by the decrease in absorbance at 240 nm ( $\epsilon = 39.4 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ ) for 2 min in a 4 mL reaction medium incubated at  $30^{\circ}\text{C}$ , containing enzyme extract, 100 mM (pH 7.0) potassium phosphate buffer and 12.5 mM hydrogen peroxide.

*Guaiacol peroxidase (POX - EC 1.11.1.7) activity*: determined as described by Urbanek *et al.* (1991), by monitoring the rate of production of tetraguaiacol by reducing hydrogen peroxide, at 470 nm ( $\epsilon = 26.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ ), for 2 min. The reaction medium, consisting of 100 mM (pH 7.0) potassium phosphate buffer,  $0.1 \mu\text{M}$  EDTA, 5 mM guaiacol and 15 mM hydrogen peroxide, was incubated at  $28^{\circ}\text{C}$ .

The experimental randomized block design consisted of three treatments and six replications. In the statistical analysis of the data, we used ANOVA, followed by Tukey's test, adopting a level of significance of 5%.

## Results and discussion

The degree of shading did not significantly affect the proportional seedling emergence, which reached 87% at all three levels of light intensity. Similarly, no differences were observed in total chlorophyll content or POX activity in red rice roots.

The length of organs of red rice seedlings was altered by light levels (Fig. 1a). A reduction in shoot length and an increase in root length were observed when the level of light intensity was increased. Seedlings exposed to a 35% level of light intensity presented longer shoots and shorter roots in comparison with those exposed to other levels of light intensity. The longer shoot in seedlings exposed to that level of light intensity is a reflection of the reduced level of solar radiation reaching the canopy and might be related to greater translocation of assimilates from the roots to the shoot of the plant. This physiological process is made evident by the reduced dry matter allocated to the roots

compared with that present in the shoot (Fig. 1a), as it is by the higher  $F_w$  in seedlings exposed to a 35% light level (Fig. 2a). According to Gobbi *et al.* (2009), lengthening of the stem and internodes is among the main morphological changes resulting from a reduction in light intensity in plants of the Poaceae family.

An increase in light intensity resulted in an increase in dry matter production, the dry mass of the shoot being superior to that of the roots at all three levels of light intensity (Fig. 1b). Seedlings exposed to a 35% level of light intensity allocated less dry matter, a process resulting from the lower collection of radiant energy by the seedling's photosynthesis apparatus and possibly from a lower rate of conversion of that energy into chemical energy, destined for plant growth and development (Marenco & Lopez 2005). Therefore, it is possible to verify the high plasticity of the genotype when exposed to a 65% light level and the state of stress when exposed to a 35% light level, as made evident by the lower production of dry matter from organs under the latter condition.

The number of tillers increased in parallel with increases in the level of light intensity (Fig. 1c), being lowest in the seedlings exposed to a 35% light level and highest in those exposed to a light level of 100%. In seedlings exposed to the two highest light levels (65% and 100%), the greater number of tillers resulted in greater shoot dry masses. The low number of tillers, in the early ontogeny of the plant, can be explained by greater carbon investment for the growth of the main stem. This process can be reflected in the delay in generating tillers, a fact that contributes to a reduction of the competitiveness of the genotype (Rigoli *et al.* 2009). The number of tillers varies from genotype to genotype, is determined by hormonal balance, and may be dependent on the light available to the plant (Valerio *et al.* 2009).

Leaf area was also affected by light level (Fig. 1d). Seedling leaf area increased at the 65% light level. The increase in the leaf area of seedlings under that condition may be related to the plant attempting to acclimate to the low light level. That process is aimed at broadening the area for the collection of radiant energy in order to increase efficiency in the production of assimilates synthesized via photosynthesis, as well as to ensure structural maintenance and adequate early seedling growth.

The  $F_w$  was highest in the seedlings exposed to a 35% light level (Fig. 2a). A higher  $F_w$  indicates greater carbon investment for leaf generation at the expense of the root system (Fig. 1b). Aumonde *et al.* (2011) reported that  $F_w$  is a component of  $F_A$  represented by the relation between the accumulation of dry matter in leaves and total dry matter. Even though the increase in the values of  $F_w$  is characteristic of high leaf growth, where photoassimilates are preferentially translocated to this organ.

The  $F_A$  represents the leaf area useful for photosynthesis. The  $F_A$  decreased as light levels increased (Fig. 2b). Therefore, the  $F_A$  value was highest in the seedlings exposed to

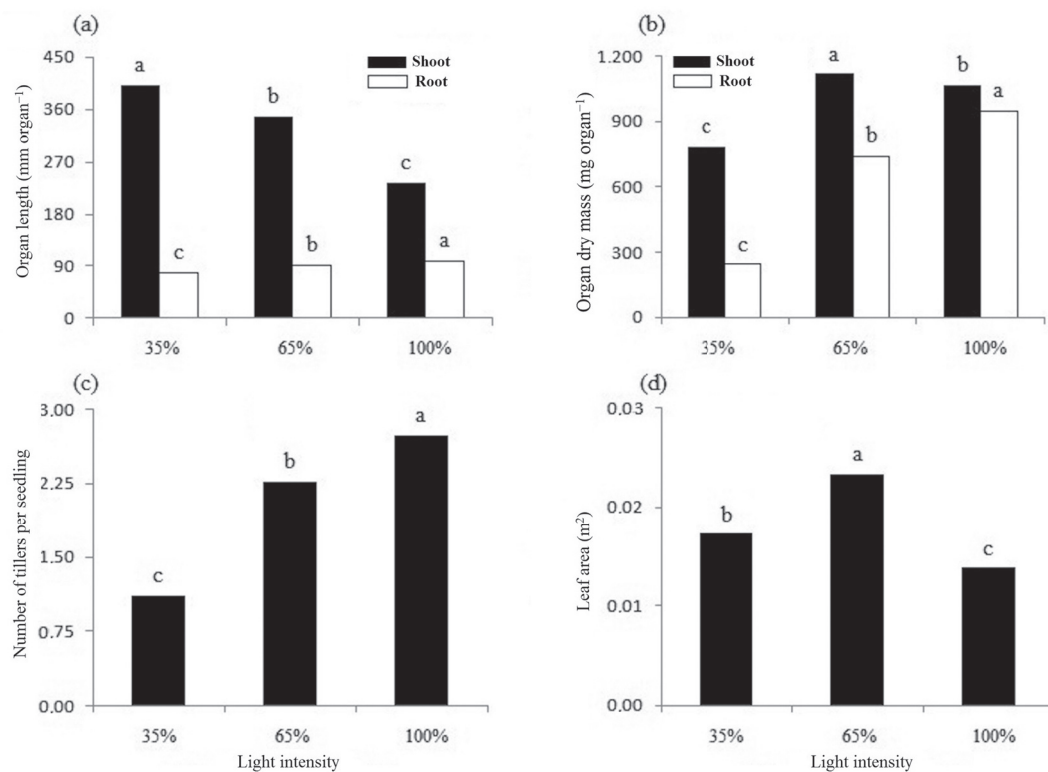
a 35% light level. Even with a smaller usable leaf area for photosynthesis, seedlings exposed to light levels of 65% and 100% presented a larger organ dry mass, possibly caused by greater efficiency in the allocation and synthesis of assimilates (Fig. 1b).

The  $S_A$  is a morphological and anatomical element of the  $F_A$  and relates leaf surface to the dry mass of the leaf itself (Aumonde *et al.* 2011). In this sense,  $S_A$  decreased with increased light levels, the highest values therefore being obtained at the two lower levels (Fig. 2c). It can be stated that seedlings exposed to a 35% light level presented larger and thinner leaves compared with those at 65% and 100% light levels, which contributed to a larger leaf area under such conditions (Fig. 2a). According to Aumonde *et al.* 2011), lower values of  $S_A$  are due to a reduction or stoppage of leaf area expansion associated with the increase in dry leaf matter. Leaf expansion under low light conditions is a compensatory measure aimed at better use of limited light availability (Lima *et al.* 2008).

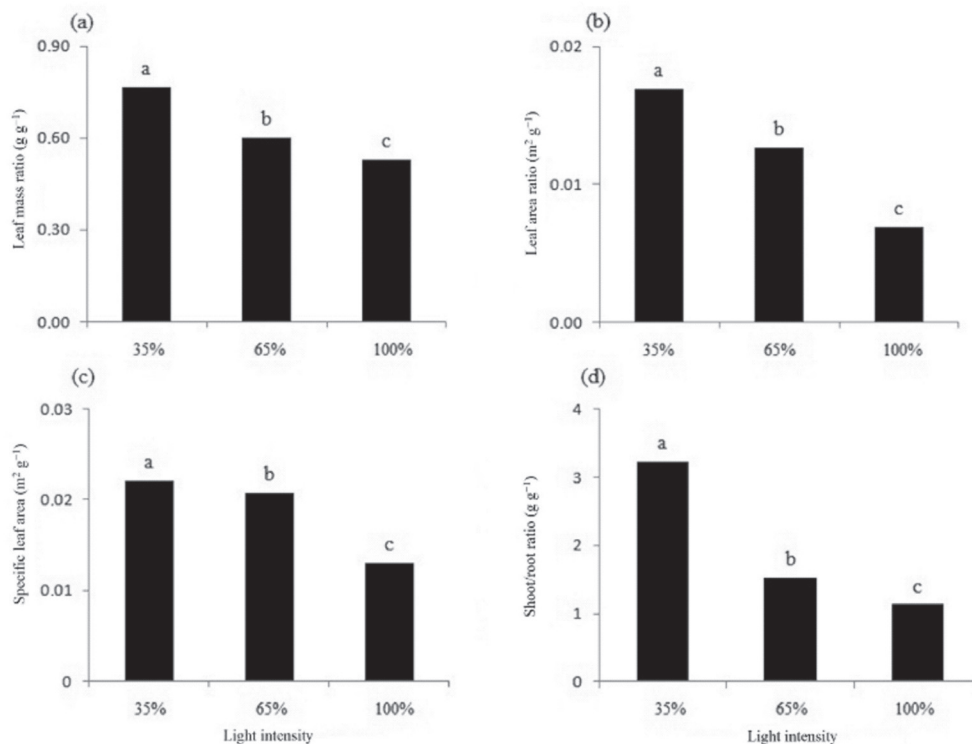
The  $P_W$  was superior in seedlings exposed to a 35% light level (Fig. 2d). A larger  $P_W$  ratio indicates greater allocation of dry matter in the shoot compared with the root system. For adequate plant development to occur, there must be a balance between the growth of the shoot and the roots. An adequate root system has greater capacity to absorb water and nutrients. In this regard, any condition that reduces

root growth can influence the competitive ability of red rice genotypes. According to Gobbi *et al.* (2009), in plants of the Poaceae family grown under reduced light levels there is also an increase in the  $P_W$  and  $S_A$  ratios, leaf blade elongation, reduced tillering and changes to the leaf inclination angle.

Chlorophyll content was affected qualitative and quantitatively by light levels (Fig. 3a). Higher levels of light intensity were found to result in increases in chlorophyll *a* content, which was highest in the seedlings exposed to a 100% light level. That might have contributed to greater capture of light energy, promoting the efficiency of the photosynthesis process. The content of chlorophyll *b* showed a tendency to decrease when the light level was raised and was therefore highest in the seedlings exposed to 35% light levels. Chlorophyll *b* captures light radiation of a different wavelength and transfers the energy to chlorophyll *a*, which effectively participates in the photochemical reactions of photosynthesis (Scalon *et al.* 2003). It should be noted that an increase in the level of chlorophyll *a*, which is more effective in capturing radiant energy for photosynthesis, provides an increase in electron transport in photosystems, resulting in the production of reactive oxygen species. According to Almeida *et al.* (2004), plants that have higher chlorophyll levels are potentially capable of achieving higher photosynthesis rates and therefore adapt better to different environmental conditions.



**Figure 1.** Length of organs (a), dry mass of organs (b), number of tillers (c) and leaf area (d) of red rice seedlings exposed to varying levels of light intensity.



**Figure 2.** Leaf mass ratio (a), leaf area ratio (b), specific leaf area (c) and shoot/root ratio (d) of red rice seedlings exposed to varying levels of light intensity.

Activity of the SOD enzyme was greatest in the leaves and roots of the seedlings exposed to 35% light levels, with correspondingly lower activity at 65% and 100% light levels (Fig. 3b). The increase in SOD activity might be due to increased production of the superoxide radical, considered the first enzyme to act on the antioxidant system, consummating the dismutation of the superoxide radical to hydrogen peroxide (Azevedo Neto *et al.* 2006; Sinha & Saxena 2006).

In the leaves of seedlings, the APX enzyme showed its greatest activity when the seedlings were exposed to a 100% light level (Fig. 3c). In the roots, APX activity was greatest in the seedlings exposed to a 65% light level. This increase in APX activity in the leaves might be related to high levels of hydrogen peroxide originating from the conversion of the superoxide radical through SOD-mediated reactions (Sinha & Saxena 2006). According to Foyer & Noctor (2000), APX is one of the main peroxidases acting in the removal of this free radical, using ascorbate to reduce hydrogen peroxide to water.

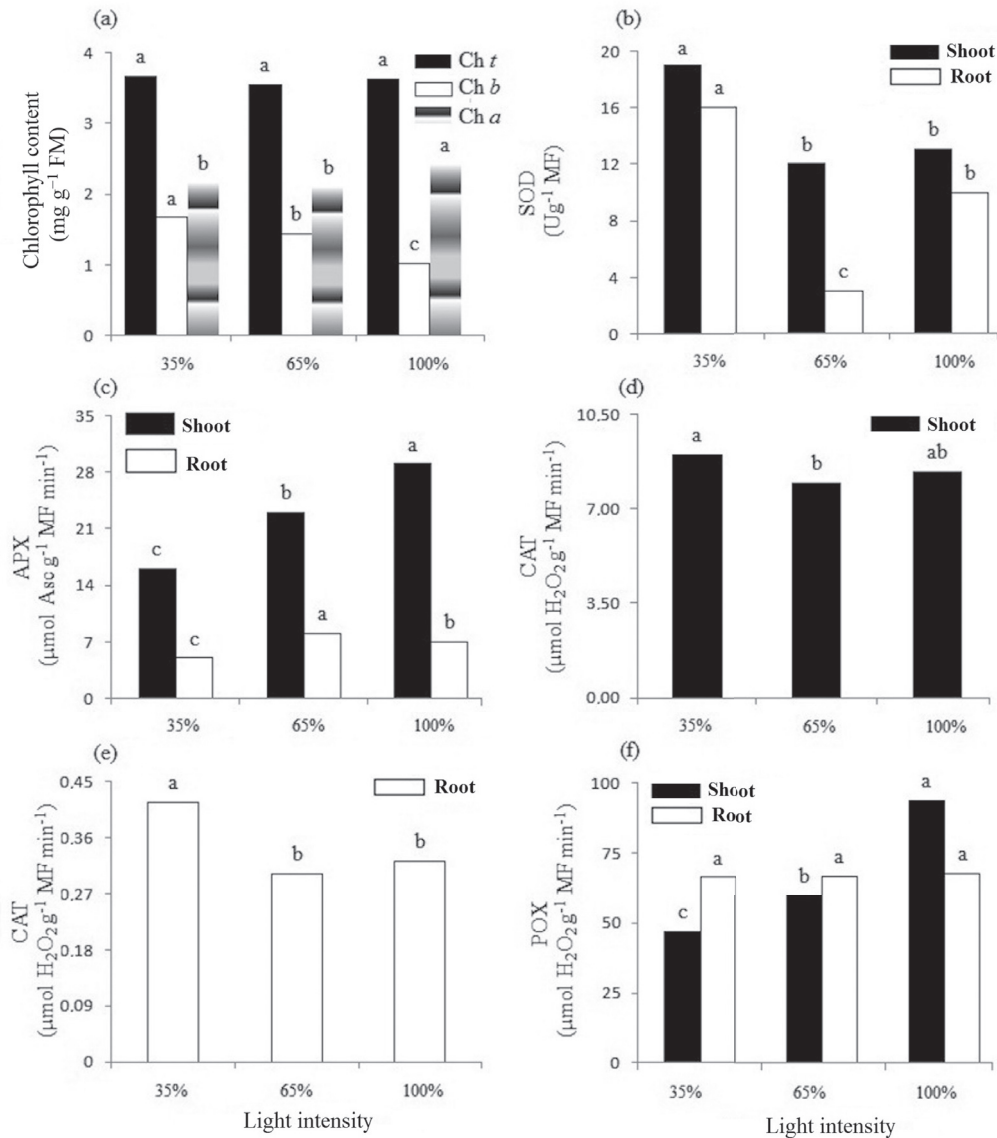
The activity of the CAT enzyme in leaves and roots changed according to the level of light intensity (Fig. 3d and 3e). The CAT activity, in both leaves and roots, was highest in the seedlings exposed to a 35% light level, whereas it was lowest in the leaves of seedlings exposed to a 65% light level and in roots of those exposed to a 100% light level. Unlike APX, CAT has a low affinity for hydrogen peroxide, which is important for the removal of hydrogen peroxide

resulting from photorespiration (Foyer & Noctor 2000) and provides evidence of the low production of hydrogen peroxide in seedlings exposed to high levels of light intensity.

The POX enzyme activity in leaves increased in parallel with increases in the level of light intensity (Fig. 3f). Therefore, the lowest POX activity was observed in seedlings exposed to a 35% light level.

In the roots and leaves of seedlings exposed to a 35% light level, after SOD, the CAT enzyme might have been the enzyme most responsible for detoxifying peroxide. However, at the highest level of light intensity (100%), the enzymes most responsible for peroxide detoxification were APX and POX. This difference among antioxidant enzymes was also observed by Azevedo Neto *et al.* (2006), who studied the effect of salt on maize genotypes, in which CAT and POX enzymes proved to be more responsible for countering the effects of salt than was APX. In the present study, the antioxidant metabolism responded differently according to the stressor (the light level).

The ability to increase SOD, APX and POX activity is essential for maintaining the balance between the formation and degradation of free radicals that are potentially harmful to plant cell systems (Sinha & Saxena 2006). This explains, in part, the higher activity of these enzymes in seedlings exposed to a 100% light level compared with those exposed to a 65% light level. It is of note that rice is a C<sub>3</sub> plant, in which photosynthesis is accompanied by photorespiration.



**Figure 3.** Chlorophyll content (a) and enzyme activity—superoxide dismutase (SOD, b); ascorbate peroxidase (APX, c); catalase (CAT, d and e) and guaiacol peroxidase (POX, f)—in red rice seedlings exposed to varying levels of light intensity. Ch t – total chlorophyll; Ch a – chlorophyll a; Ch b – chlorophyll b; FM – fresh mass.

Therefore, when exposed to high levels of light intensity, rice not only loses carbon but also presents an increase in the production of reactive oxygen species (Apel & Hirt 2004).

On the basis of the overall results and taking the attributes of early growth into consideration, we can affirm that the seedlings responded most effectively to an intermediate level of light intensity (65%). Similar results were obtained in relation to the antioxidant metabolism through low SOD enzyme activity, an enzyme at the first line of defense, responsible for detoxification of the superoxide radical, which is converted to hydrogen peroxide. Therefore, we can infer that intermediate light conditions are the most favorable environment for the early growth of red rice seedlings.

Extremes of light intensity act as major stressors, which can cause inefficient antioxidant metabolism, resulting in increased production and accumulation of free radicals. That process has negative effects on the characteristics of early growth of red rice seedlings.

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