Australian Journal of Crop Science AJCS
AJCS 9(4):344-354 (2015) ISSN:1835-2707

Physiological responses of rice cultivars exposed to different temperatures and flood depths in a water seeded system

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Abstract

Temperature and flood depths influence the growth and development of irrigated rice. The objective of this study was to evaluate the initial response of two rice genotypes on oxidative stress, growth and nitrogen accumulation of rice seedlings under different temperatures and flood depths in a water seeded system. The study was conducted in 2012 using a phytotron chamber. Treatments were a combination of two air temperatures regimes (17 and 20°C), two rice genotypes (IRGA 425 and Epagri114) and three flood dephs (1, 5 and 9 cm). The results showed that temperature affected seedling performance, with greater dry mass for roots and shoots, as well as greater nitrogen accumulation at 20°C at a flood depth of 1 cm for both genotypes. The H_2O_2 concentration in the root increased with increasing flood depth, with a significantly greater increase for the 114 Epagri genotype. The activity of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) varied depending on the genotype, with SOD being the most expressive, increasing its activity with increasing flood depth. Chlorophyll and carotenoid contents decreased significantly with increasing flood depth, with less interference for IRGA 425. Temperature and flood depth affected nitrogen absorption, dry matter accumulation and oxidative stress in rice seedlings in the system tested. The lowest stresses were observed in rice plants subjected to the flood depth of 1 cm at 20 °C, and cultivar IRGA 425 was more tolerant to increased depth of water when compared to cultivar Epagri 114.

Keywords: Cold; Flooding depth; Nitrogen; *Oryza sativa*; Oxidative stress.
Abbreviation: H_2O_2_hydrogen peroxide; SOD_ superoxide dismutase; CAT_ catalase; APX_ ascorbate peroxidase; DAS_days after seeding; S_ shoots; R_ roots; MDA_malondialdehyde.

Introduction

Irrigated rice is an important crop worldwide and a staple food for a large part of the population (Kanno et al., 2009). Rice yield is influenced by either high or low air temperature extremes (Gammulla et al., 2010). Low air temperature reduces the vegetative growth of the rice plant and increases grain sterility, decreasing grain yield by 30-40% in some parts of the world (Gammulla et al., 2010). One of the most important ways to increase the potential grain yield of rice crops is to match the period of greater crop responsiveness with the occurrence of higher appropriate solar radiation and temperature in the region (Freitas et al., 2008; Katsura et al., 2008; Safdar et al., 2008; Lack et al., 2012). However, for this to occur, early seeding is held in the period when air and soil temperatures are low, causing stress and a number of morphological and physiological changes in the crop (Ohsumi et al., 2012). Thus, the biomass production (Ohsumi et al., 2012), as well as the absorption and use of nitrogen, which is one of the determining factors in crop yield (Azam et al., 2003; Kanno et al., 2009), may be impaired. An alternative to minimize stress caused by low temperatures at the early development stages of rice crops is to use a water seeded system, where growth and early development of the radicle and/or shoot occur under the water layer. Thus, the properties of water such as specific heat and latent heat, contribute to buffering temperature fluctuations (Taiz and Zeiger, 2006). However, in this system, there is a demand for genotypes with improved early development, because the performance of rice seedlings under anaerobic conditions depends on seed vigor and seedling capacity to degrade the reserve substances of the seed and convert them into new biomolecules, allowing them to grow until they reach the water surface (Wielewicki and Barros, 2003). In addition, in a water seeded system, the amount of water (flooding) is one of the factors causing stress (Mittal et al., 2009), because the O_2 concentration is low in this environment, which leads to anoxia (no O_2) or hypoxia (low O_2), generating toxic levels of H_2O_2 (Damanik et al., 2012). Thus, morphological changes may occur, such as stem elongation of plants and chlorosis in some rice genotypes that are not very tolerant to flooding (Kawano et al., 2002). According to Kocsy et al. (2011), low temperature stress causes significant yield loss in cereals. Moreover, this kind of stress may increase the production of reactive oxygen species (ROS) (Upadhyaya et al., 2007; Gill and Tujeta, 2010; Ranawake et al., 2012) and consequently damage membrane lipids, proteins, photosynthetic pigments and nucleic acids, which may result in cell death (Mittler 2002; Gill and Tujeta 2010). There are several established mechanisms that can reduce or prevent oxidative stress (Bonnecarrère et al. 2011). The antioxidant defense system is responsible for maintaining a balanced production of ROS. The system may be comprised of enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and

ascorbate peroxidase (APX) (Ella et al., 2003) and non-enzymatic ones such as carotenoids, which play a significant role in the photosynthetic system of plants (Salguero et al., 2003). This study evaluated the initial response of two rice genotypes at different air temperatures and flood depths in a water seeded system by verifying oxidative stress parameters, growth and nitrogen accumulation of seedlings. This is the first report to use a combination of different air temperatures and flood depths in a water seeded system to assess effective rice seedling establishment.

Results and discussion

Effect of air temperature on water and soil temperature

At the two target levels of air temperatures (17 and 20°C), water and soil temperatures were influenced by flood depth during the 25 days of the experiment (Fig 1 A, B). Soil temperature was always higher compared to water temperature at the same flood depth. Furthermore, in general, an increase in flood depth reduced both water temperature and soil temperature. The greatest reductions occurred in water temperature, with an average reduction of 0.4°C when the depth increased from 1 cm to 9 cm during the entire period.

Effect of air temperature and flood depth on root dry mass

There was a triple interaction among air temperature, cultivar and flood depth. As shown in Fig 2 A and B, root dry mass of cultivars IRGA 425 and Epagri 114 was influenced by temperature and flood depth, indicating sensitivity of the root system of these cultivars to the factors tested. In general, there was increased production of root biomass at the depths of 1 and 5 cm at 20°C compared to 17°C for both cultivars. A linear reduction of root dry mass occurred with increased flood depth for cultivars IRGA 425 and Epagri 114, at 17 and 20°C, respectively. These results are in agreement with Broch et al. (1997), who found a reduction in root dry mass as well as root and shoot length with increased flood depth at 21 days after seeding when assessing levels of flood depth (0, 1.5 and 3.0 cm) in a water seeded system with BR-IRGA 410. According to these authors, changes in root and shoot development can be explained by the reduced concentration of oxygen resulting from lack of O_2, which directly produces many biochemical reactions, and by the stress generated by water as a physical barrier to the growth of seedlings. However, in the present study, this reduction was more pronounced at the air temperature of 20°C, and higher root dry mass production was observed at the depth of 1 cm. Every plant species has a set of particular requirements for temperature, which enable proper growth and development. Many species, especially those which are native to hot habitats, show symptoms of injury when exposed to low temperatures (Kaniuga, 2008). These plants, including maize (*Zea mays*), soybean (*Glycine max*), cotton (*Gossypium hirsutum*), banana (*Musa sp.*) and rice (*Oryza sativa*), are generally sensitive to temperatures around 15°C. At a temperature of 20°C, cultivar IRGA 425 presented decreased root dry weight with increasing flood depth. This reduction was lower when compared to that of Epagri 114, showing that IRGA 425 was less sensitive to flood depth. At 17°C, however, there was less influence of flood depth on the root dry mass of the cultivars, and IRGA 425 presented the opposite behavior, showing greater reduction than Epagri 114, which showed no significant changes in biomass. According to Kocsy et al. (2011), exposure to cold, with or without freezing, induces oxidative stress. Symptoms of injury induced by cold stress in plants occur in the first hours of exposure to low temperatures (Bonnecarrère et al., 2011). However, this period varies across species and genotypes, depending on their sensitivity to cold stress. Several phenotypic symptoms in response to cold stress include reduced leaf expansion, reduced root biomass, wilting, chlorosis (yellowing of leaves) and necrosis, in extreme cases. Low temperature also seriously hampers the reproduction and development of plants. Because increased flood depth resulted in reduced water and soil temperatures (Fig 1 A, B), it can be suggested that growth responses are an additional effect of cold stress; therefore, according to Ohsumi et al. (2012), low temperature not only reduces biomass production but also reduces rice grain yield as a result of the morphological and physiological changes that occur.

Effect of air temperature and flood depth on shoot dry mass and plant nitrogen accumulation

An interaction was found between the factors temperature and flood depth for shoot dry mass (SDM) and accumulated nitrogen (AN) per plant. Air temperature and flood depth not only interfered with root dry weight but also negatively affected shoot dry mass and nitrogen accumulation in the tissue of the cultivars (Fig 2 C and D, respectively). In general, higher production of shoot mass and greater nitrogen accumulation occurred at 20°C. According to Kanno et al. (2009), biomass production is dependent on the availability of photoassimilates produced and the efficiency of their use for growth, in addition to factors such as increased CO_2 assimilation, which may be due to the increase in leaf area and relative growth at suitable temperatures. In another study, the same authors evaluated biomass production and rice plant growth during the vegetative phase under three different night temperatures (17, 22, and 27°C), with a constant temperature of 27°C during the day. They found greater accumulation of sucrose and starch in plants under low temperature; according to Ohashi et al. (2000), this is indicative of the inefficient use of assimilates for plant growth. Another important point is that the greater accumulation of nitrogen observed in plant tissues at 20°C may have contributed to the higher shoot dry matter production in the present study. Additionally, at 20°C, there was a linear reduction in dry matter production and nitrogen accumulation with increasing flood depth. The depth of 1 cm provided greater shoot dry mass and nitrogen accumulation in the tissues. Interestingly, at 17°C flood depth did not affect these parameters. Cold stress effects include reduction of metabolic activity (Chinnusamy et al., 2007), which results in lower biomass production (Ohsumi et al., 2012), and this may be partly associated with the lower photosynthetic activity (Bagnall et al., 1988). A study by Bonnecarrère et al. (2011) evaluated two genotypes of japonica rice for tolerance to low temperatures, and a decrease was observed in the fresh weight of the genotypes at 10°C compared to that observed at 28°C. Under field conditions, Ohsumi et al. (2012) investigated two seeding dates (during the same season), one in which the average air temperature was 16.1 and 15.1°C and the other with average temperatures of 19.8 and 19°C. Based on that study, the parameters associated with number of leaves, shoot length and dry weight were higher during the period when temperatures were higher. According to the authors, the decrease in the shoots of plants grown at lower temperatures (16.1 and 15.1°C) implies that plants have a lower tissue volume to store photoassimilates. In addition to

Table 1. Total chlorophyll at 25 days after seeding (DAS), depending on air temperatures and irrigated rice cultivars. Santa Maria, RS. 2013.

	Cultivars	
	IRGA 425	Epagri 114
17	1.167 NS a*	1.217 a
20	1.084 NS b	1.027 b
Mean	1.125	1.122
CV%	6,49	

* means not followed by the same lowercase letter in the column and the same uppercase letter in the row, does not differ by Tukey's test at 5% probability; NS - non-significant.

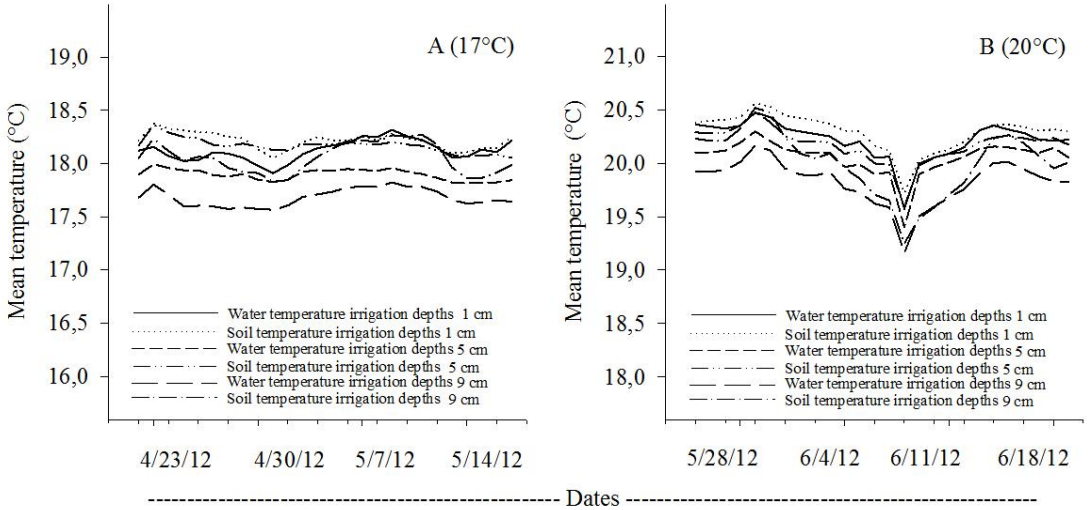

Fig 1. Water and soil temperatures at 17 and 20°C at flood depths of 1, 5 and 9 cm. Santa Maria, RS. 2013.

the production of biomass, mineralization and nitrogen availability for the plants are considerably dependent on the temperature of the rhizosphere (Azam et al., 2003). According to these authors, there may be genotypic differences for the effect of temperature in the root zone in terms of root development, nutrition and nutrient cycling. Shimono et al. (2012) evaluated nitrogen absorption in rice at different water temperatures (16 to 25 °C) and found an increase in the rate of nitrogen absorption with increasing water temperatures. They observed that low temperature affects the rate of nitrogen absorption, corroborating the data of this study. Accordingly, this reduction may have been decisive for the lack of differentiation in growth response across flood depths at 17°C, because reduced growth was observed even at the lowest flood depth. Temperature, coupled with flood depth, may account for the lower accumulation of nitrogen in shoots and, more markedly, in the root system.

Effect of air temperature and flood depth on root/shoot ratio

For root/shoot ratio (RSR), there was a triple interaction among air temperature, cultivar and flood depth. The biomass ratio (R/S) (Fig 2 E, F) showed a similar behavior to that of root dry mass. At 20°C, both rice cultivars showed a linear decrease in the R/S ratio with increasing flood depth. These data show that the partition of reserves and production of photoassimilates of these rice cultivars are sensitive to increased flood depth, which results in less biomass allocated to roots compared to shoots. This behavior may be partly associated with ethylene production, which is induced by submerging (Kawano et al., 2002). This can result in morphological and physiological changes, depending on how much the cultivar tolerates submersion. On the other hand, at the temperature of 17 °C, the flood depth did not affect the biomass ratio of IRGA 425, while cultivar Epagri 114 showed a quadratic behavior with a higher R/S ratio at flood depths of 5 and 9 cm. Furthermore, there was a greater R/S ratio at 20 °C compared to 17 °C at the depths of 1 and 5 cm for cultivar IRGA 425. Responses to stress by submersion may vary with the genotype and species tested, and there may also be common responses to genotypes and species. These types of stress may go unnoticed during the plant cycle, and they are relevant components of the metabolism; however, if stress exceeds the capability of the plant to respond via the antioxidant system and does not follow the normal cell cycling, metabolic structural damage is observed, and cell division, cellular membranes and organelles are negatively affected (Damanik et al., 2012).

Shoot and root H_2O_2 concentration

There was a triple interaction among temperature, cultivar and flood depth for: H_2O_2 concentration in shoots. However, for H_2O_2 concentration in the roots, there was a double interaction between cultivar and flood depth. H_2O_2 concentration in shoots of cultivar IRGA 425 at 17 °C was not altered by variations in flood depth (Fig 3 A, B). However, at 20 °C, there was a linear reduction of H_2O_2 concentration (11%) with increasing flood depth, indicating that this cultivar is more tolerant to flood depth with regard to shoot growth. On the other hand, cultivar Epagri 114 showed a 60% increase in H_2O_2 concentration in the shoot as flood depth increased from 1 to 9 cm at 17 °C, but no influence was observed at 20 °C. In the roots (Fig 3C), both cultivars showed increased H_2O concentration with increasing flood depth. The greatest change was observed for Epagri 114, with a 15% increase as flood depth increased from 1 to 9 cm. Thus, it appears that the root system is more sensitive to increased flood depth than are the shoots. In general, it was also observed that cultivar Epagri 114 showed higher H_2O_2

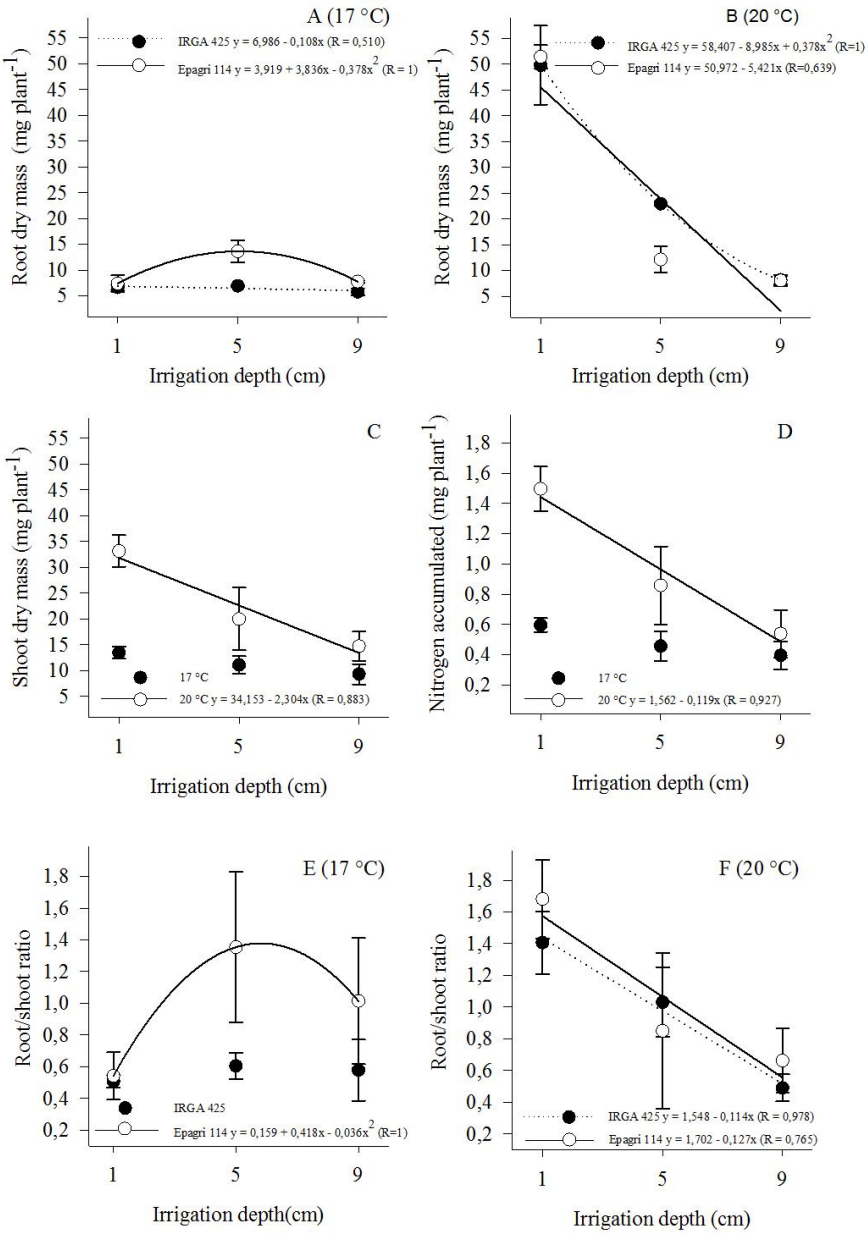

Fig 2. Root dry mass at 25 days after seeding (DAS) at 17°C (A) and 20°C (B); shoot dry mass at 25 DAS (C), accumulated nitrogen per plant in the shoots at 25 DAS (D); and root/shoot ratio at 25 DAS at 17°C (E) and 20°C (F) as a function of rice cultivar and flood depth. Santa Maria, RS. In 2013.

concentrations in roots than IRGA 425 at all flood depths, indicating its greater sensitivity. Increased H_2O_2 concentration has been observed not only at temperatures lower than the optimum (25°C) for growth of rice but also at temperatures higher than ideal. Bhattacharjee (2012), evaluating the effect of air temperatures (8, 25 and 40°C) on two rice cultivars for 24 hours, reported increased H_2O_2 concentration in roots and shoots at extreme temperatures (40 and 8°C) compared to the control (25°C). Matsumura et al. (2002), also studying rice, evaluated the effect of exposure to temperatures ranging from 5 to 25°C. In that study, H_2O_2 concentration increased in leaves of rice plants temporarily exposed to low temperature, and this resulted in an increase in senescent leaves. According to these authors, exposure to low temperatures for a long period of time may lead to H_2O_2 accumulation and disturbance of the redox state equilibrium. Zhang et al. (2012) also reported morphological and physiological changes in the root system of cucurbits (*Cucumis sativus* L) induced by low temperatures, including a reduction in fresh weight, and increased H_2O_2 concentration at 14 °C compared to 24°C. According to these authors, ROS are produced at low levels in organelles such as chloroplasts, mitochondria and peroxisomes under optimal growth conditions, whereas their production is markedly increased during stress. According to Cheng et al. (2007), the function of H_2O_2 is to mediate signal transduction in response to biotic and abiotic stresses in plant cells. Moreover, rice crops are sensitive even to mild cold stress, particularly at the early stages of seedling establishment, and japonica cultivars are generally more tolerant than most indica cultivars (Cheng et al., 2007).

Lipid peroxidation (MDA concentration)

There was a triple interaction among temperature, cultivar and flood depth for lipid peroxidation (MDA concentration).

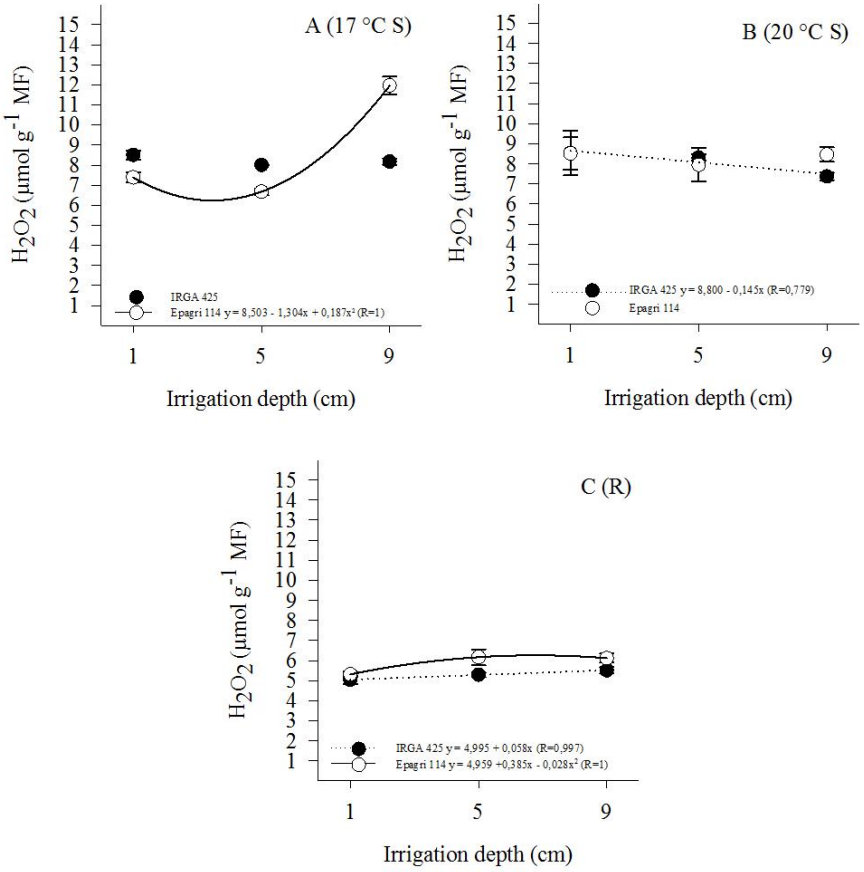

Fig 3. H_2O_2 concentration in shoots (S) (A, B) at 17°C and 20°C and H_2O_2 concentration in roots (R) (C) at 25 days after seeding (DAS) as a function of rice cultivar and flood depth. Santa Maria, RS. 2013.

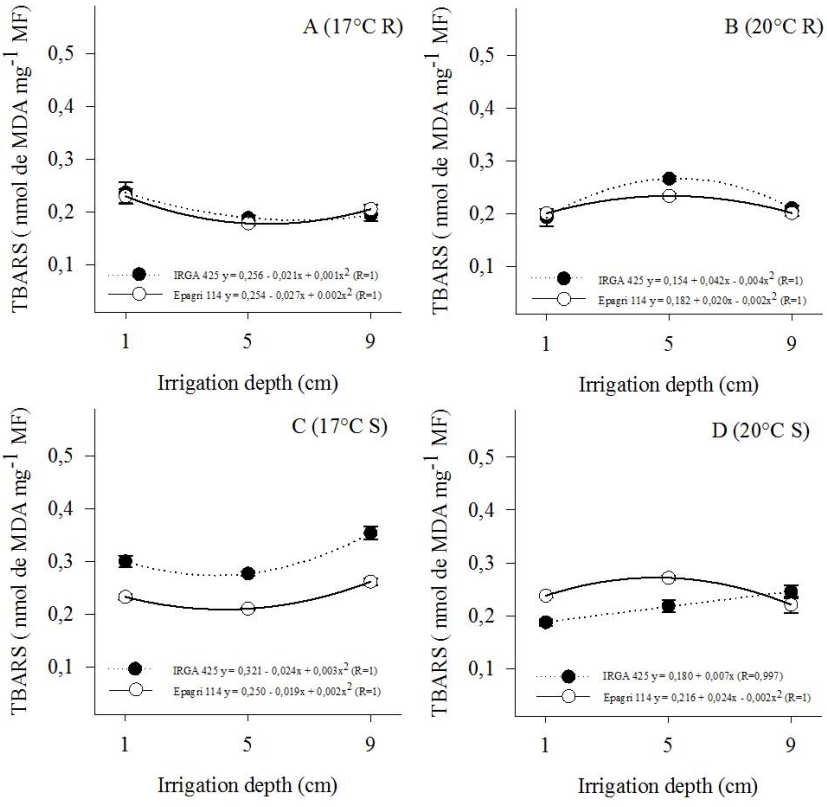

Fig 4. Lipid peroxidation in roots (R) (A, B) and shoots (S) (C, D) at 17°C and 20°C at 25 days after seeding (DAS) as a function of rice cultivar and flood depth. Santa Maria, RS. 2013.

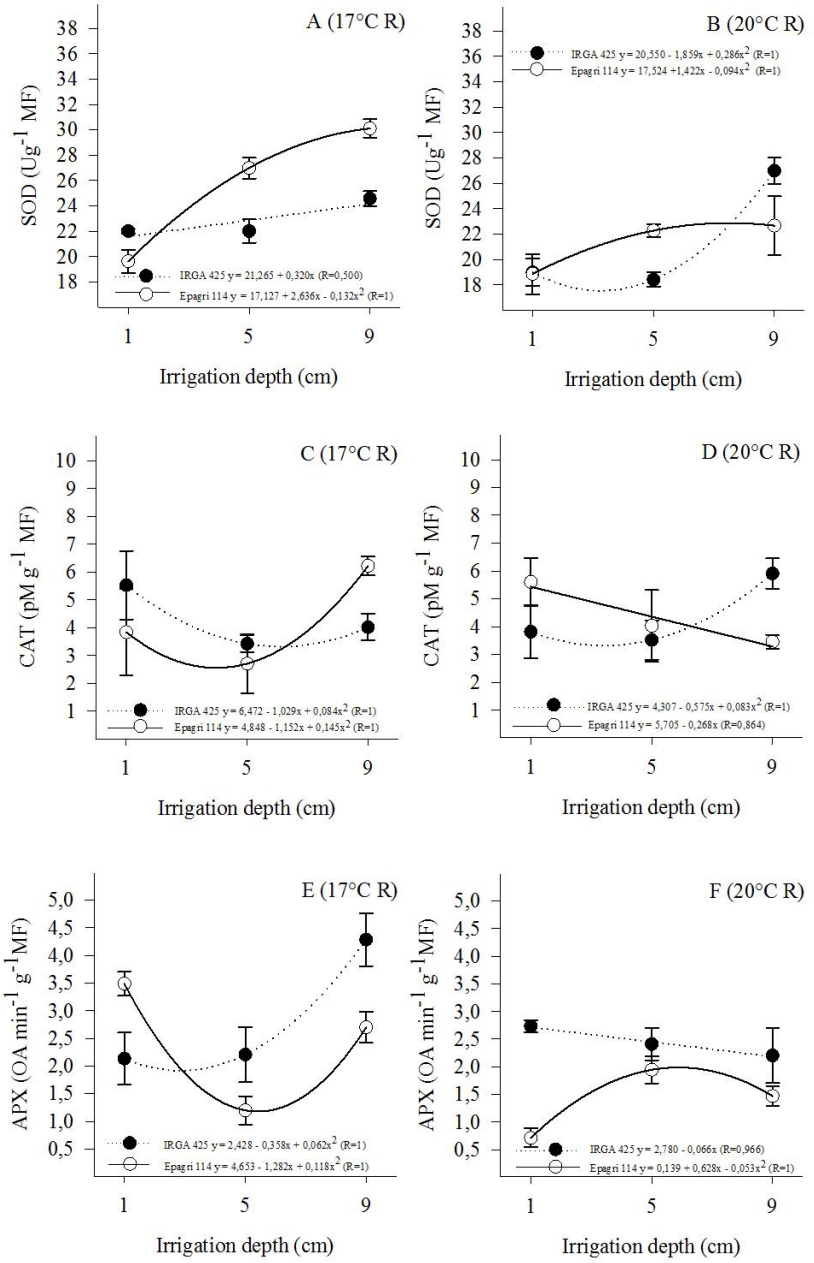

Fig 5. SOD (A, B), CAT (C, D) and APX (E, F) activity in roots (R) at 17°C and 20°C at 25 days after seeding (DAS) as a function of rice cultivar and flood depth. Santa Maria, RS. 2013.

In the roots, there was a slight reduction in MDA concentration for both IRGA 425 and Epagri 114, with increasing flood depth at 17 °C (Fig 4 A). When the temperature increased to 20 °C (Fig 4B), there was a smaller effect of flood depth on MDA concentration, with the highest concentration observed at the flood depth of 5 cm, where MDA levels were 38% and 16% higher in the tissue of roots as compared to that observed at the depth of 1 cm for cultivars IRGA 425 and Epagri 114, respectively. The increased MDA levels in roots might be due to the increased H_2O_2 concentration, resulting in increased lipid peroxidation (Ella et al., 2003; Upadhyaya et al., 2007). This would account for the reduced root growth of the two cultivars at increased flood depths. Lipid peroxidation is considered to be one of the most harmful processes in living organisms, indicating damage occurring in cell membranes (Gill and Tujeta, 2010). Furthermore, lipid peroxidation may have damaged the chloroplast by inhibiting the synthesis of chlorophyll and, thus, photosynthesis (Ella et al., 2003).

In the shoots (Fig 4C), unlike that which occurred in the roots, at 17°C there was an increase in MDA concentration with increasing flood depths (from 1 to 9 cm). The increase, by 18% for IRGA 425 and by 12% for cultivar Epagri 114, was also due to the higher production of H_2O_2. In this study, no significant differences were observed in MDA concentration in relation to changes in temperature. However, some studies have shown increased MDA concentration with decreasing temperature (Shi et al., 2006; Bonnecarrère et al., 2011; Kim and Tai, 2011). At 20°C (Fig. 4 D), this behavior was also observed, with an increase of 31% for IRGA 425. For Epagri, an increase of 14% in MDA was observed at a depth of 5 cm when compared to that observed at 1 cm.

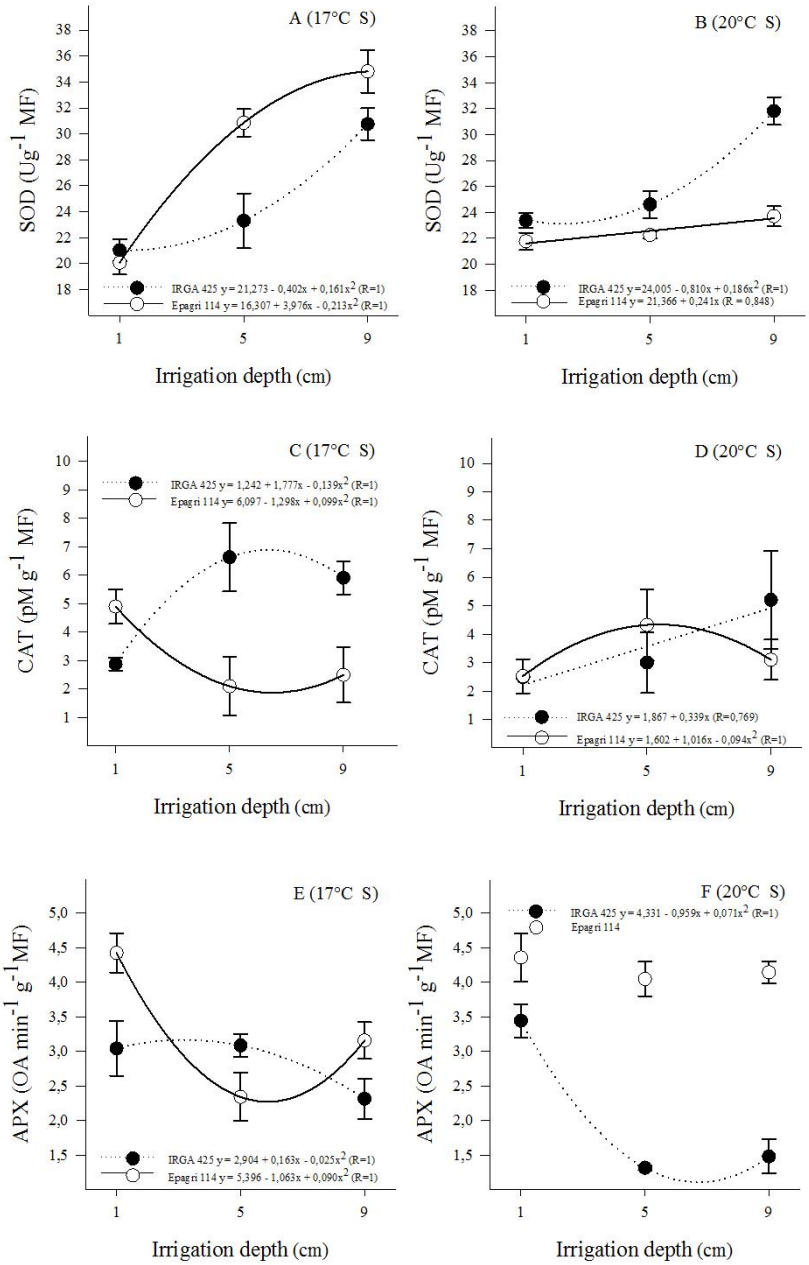

Fig 6. SOD (A, B), CAT (C, D) and APX (E, F) activity in shoots (S) at 17°C and 20°C at 25 days after seeding (DAS) as a function of rice cultivar and flood depth. Santa Maria, RS. 2013.

Antioxidative enzyme activities

There was a triple interaction among temperature, cultivar and flood depth for SOD activity, CAT activity and APX activity in roots and shoots. SOD activity in roots (Fig 5 A, B) increased with increasing flood depth at 17 °C and 20 °C for both cultivars. For cultivar IRGA 425, the increase was of 53 and 20% at 17 °C and 20°C, respectively, while for Epagri 114 the increase in SOD activity was of 12 and 42% at 17°C and 20 °C, respectively. In general, there was increased SOD activity at the lower temperature (17 °C). SOD activity in the shoots (Fig 6 A, B) was similar to SOD activity in the roots. For both cultivars and air temperatures, the highest SOD activity occurred at the depth of 9 cm. At this flood depth, SOD activity for Epagri 114 was 73 and 8% higher when compared to 1 cm, for 17°C and 20°C, respectively. For IRGA 425, the increase was of 46 and 36% at 17°C and 20°C, respectively. Similar to that observed in the root, SOD activity was also higher at the lower temperature. Based on the results shown in Fig 6 A and B, it can be concluded that cultivar Epagri 114 shows greater sensitivity to flood depth at the lower temperature (17 °C) when compared to IRGA 425. However IRGA 425 shows a similar behavior at both temperatures, but also presents higher SOD activity at a flood depth of 9 cm. CAT activity in roots (Fig 5 C, D) presented variations in accordance with the cultivar and temperatures evaluated. At 17°C, CAT activity for the cultivar IRGA 425 was 37% higher at 1 cm than at 9 cm of depth. However, the same cultivar had an opposite behavior at 20°C, where there was higher CAT activity at the greatest flood depth of 9 cm. Considering the temperature, cultivar Epagri 114 also behaved differently in terms of CAT activity, which was 62% higher at the depth of 9 cm, when compared to the depth of 1 cm at 17ºC. However, at 20 °C, this cultivar showed higher

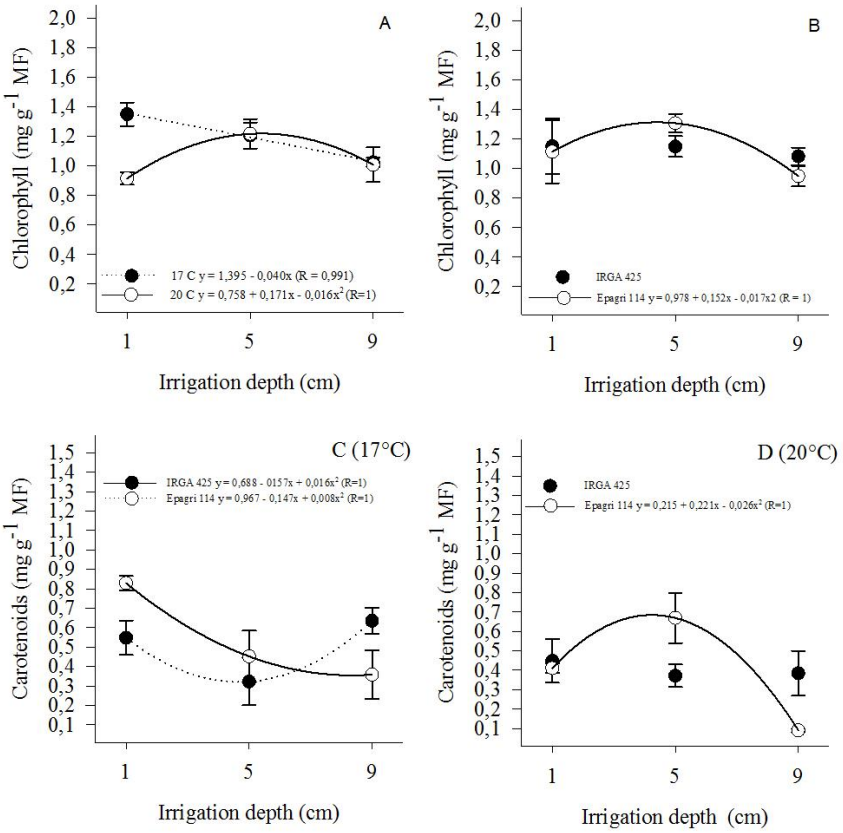

Fig 7. Concentration of total chlorophyll (A, B) and carotenoids at 17°C (C) and 20°C (D) at 25 days after seeding (DAS) as a function of rice cultivar and flood depth. Santa Maria, RS. 2013.

CAT activity at the depth of 1 cm, showing a linear decrease with increasing flood depth. In the shoots (Fig 6 C, D), for both temperatures, cultivar IRGA 425 showed an increase in CAT activity with increasing flood depth. However, Epagri 114 showed higher CAT activity at the smallest flood depth (1 cm) at the lower temperature (17°C), and there was increased activity with increasing temperature and depth, with 71% higher activity at 5 cm compared to 1 cm. It was also observed that, in general, at the lower temperature, CAT activity was higher for IRGA 425, but there were no differences between the cultivars at 20°C. At 17 °C, APX activity in roots (Fig 5 E, F) for cultivar IRGA 425 was 100% higher at 9 cm compared to the depth of 1 cm. However, at 20°C, higher APX activity was observed at 1 cm, with a subsequent reduction with increased flood depth. Epagri 114 showed a different behavior than IRGA 425, with a 30% increase of APX activity at 1 cm (17°C), than at the highest depth (9 cm), and increased activity with increasing temperature and flood depth. In the shoots (Fig 6 E, F), cultivars IRGA 425 and Epagri 114 generally showed higher APX activity at the lower flood depth (1 cm). It was also observed that Epagri 114, except for the depth of 5 cm at 17 °C, presented higher APX activity compared to IRGA 425 at all flood depths and temperatures. Thus, it appears that there was variation between the cultivars with regards to the assessed antioxidant enzymes (SOD, CAT and APX). However, SOD stood out as an important enzyme in the defense system of rice, clearly demonstrating the effect of temperature and flood depth in irrigated rice crops. This is due to the fact that SOD is the first enzyme to act in the detoxification process, converting the superoxide anion radical into hydrogen peroxide (Damanik et al., 2012), while CAT and APX catalyze the reduction of hydrogen peroxide into water and oxygen (Ella et al. 2003). According to Damanik et al. (2012), an efficient combination of SOD and CAT can minimize the effects of oxidative stress, playing an important role in the regulation of ROS. According to Saher et al. (2005), SOD is a key enzyme in the response to anoxia, which can result from the formation of toxic levels of H_2O_2. It is well established that various environmental stresses often increase the generation of ROS, with SOD playing an important role in plant tolerance to stresses, considered the first mechanism of defense against the toxic effects of high levels of ROS (Gill and Tujeta, 2010). According to Bonnecarrère et al. (2011), high SOD activity may help to establish tolerance during the early hours of cold stress. In other species, such as *Cucumis sativus* L., Zhang et al. (2012) found an increase in SOD activity at low temperatures, with no effect on CAT and APX. Bonnecarrère et al. (2011) reported that several studies have shown differences in the behavior of SOD, CAT and APX after plant exposure to low temperatures, both in tolerant and susceptible genotypes, which implies that a response of these antioxidant enzymes may or may not reflect the mechanisms involved in tolerance or susceptibility to cold temperatures. In addition to antioxidant enzymes, other studies in rice have shown more than 1900 proteins expressed at a given temperature (12 and 20°C or 36 and 44°C), 850 of which were responsive to both temperature extremes (Gammulla et al., 2010).

Chlorophyll and carotenoid

For chlorophyll in plant shoots there were double interactions for temperature and cultivar; temperature and flood depth; and cultivar and flood depth. For carotenoids in the shoots there was a triple interaction among temperature, cultivar and

flood depth. For total chlorophyll content (Fig 7 A, B), cultivar IRGA 425 presented a higher content at the lowest flood depth (1 cm) at 17 °C, with reducing content as flood depth increased. At 20 °C, there was no change in chlorophyll content with increasing flood depth for this cultivar. For Epagri 114, at the lower temperature, there was a small increase in chlorophyll content with increasing flood depth, but there was a reduction in chlorophyll content with increasing flood depth at 20 °C. Between the cultivars (Table 1), there was no difference for either temperature, but there was lower chlorophyll content at the higher temperature (20 °C). The concentration of carotenoids was also affected by the change in flood depth, with lower concentrations at the highest depth (9 cm), at both temperatures, for cultivar Epagri 114 (Fig 7 C, D). For the same cultivar, there was a reduction of 30 and 16% in the concentration of carotenoids at 9 cm when compared to the depth of 1 cm at 17°C and 20°C, respectively. On the other hand, IRGA 425 suffered less interference from temperature and flood depth. The lower concentrations of chlorophyll and carotenoids with increasing flood depth may have resulted from the increased H_2O_2 concentration. Upadhyaya et al. (2007) evaluated the effect of various H_2O_2 concentrations (0 to 1 mM) in the primary leaves of rice, and found lower content of carotenoids and chlorophyll with an increase in H_2O_2 concentration.

Carotenoids b-carotene and zeaxanthin and tocopherols play an important photoprotective role, either by dissipating excess energy as heat or cleaning ROS (Gill and Tujeta, 2010). According to these authors, there are over 600 carotenoids in nature. Carotenoids such as neoxanthin and lutein have been associated with the cleaning processes of ROS (Bonnecarrère et al., 2011). A study by Shi et al. (2006) evaluated the effects of low temperature on the pigment content and antioxidant activity on flag leaves of two varieties of rice. They found a reduction in the content of photosynthetic pigments (chlorophyll and carotenoids) with decreasing temperatures. However, the lower chlorophyll content observed in the present study for the higher temperature (20 °C) may be explained by the dilution effect resulting from higher plant growth, observed by measuring dry mass at this temperature compared to 17 °C (Table 1). The decrease in carotenoid content with increasing flood depth has important consequences for plants, such as slower growth and development, or lower dry matter accumulation, as observed in this study. This is because carotenoids are important pigments for absorbing light during photosynthesis, and their reduction results in a reduction in photosynthetic activity and therefore, in growth and development.

Summary

It was found that air temperature and flood depth are important factors in rice production as they may cause stress in plants with consequences for nitrogen absorption in plant growth and development. However, this behavior was observed in plants at 25 DAS and at constant temperature conditions. After this period, the presence or absence of plant recovery will depend on exposure conditions, which can result in yield loss. The resulting effect will depend on the cultivar, because stress response varies with the tolerance of the plant to air temperature and flood depth. In this study, the lowest flood depth (1 cm) and the highest temperature (20°C) resulted in higher plant performance due to increased nitrogen absorption and dry matter accumulation in plants. Furthermore, cultivar IRGA 425 was found to be more tolerant to these factors when compared to Epagri 114.

The lower performance of rice plants observed in this study is reflective of oxidative stress, which was identified by the enzymatic and non-enzymatic defense systems of the plants. These stresses are caused by low temperature (17°C) and great flood depth (9 cm). As a management strategy, lower flood depth should be used for rice at the beginning of seeding or when the temperature is low during this period. However, this depth must be great enough to provide enhanced nitrogen absorption and, thus, increased plant growth and efficient weed control.

Materials and Methods

Experimental design, plant material and growth conditions

The study was conducted in 2012 in a phytotron chamber at the Federal University of Santa Maria (UFSM), in the state of Rio Grande do Sul (RS), Brazil. The experimental design was completely randomized in a factorial scheme (2 x 2 x 3), with four replications. The A factor consisted of air temperatures of 17 and 20 °C, the C factor, of irrigated rice cultivars IRGA 425 and Epagri114, and the D factor, flood depths of 1, 5 and 9 cm. The two cultivars were chosen because they are the most widely used in the water seeded system. IRGA 425 presents a normal growth cycle while Epagri 114 presents a late cycle. The experimental units consisted of plastic trays with an area of 0.129 m^2. The substratum was unfertilized soil with the following physico-chemical composition at the time of seeding: water pH (1:1) = 5.6; Ca $cmol_c$ DM^{-3} = 3.3, Mg $cmol_c$ dm^{-3} = 1.0; Al $cmol_c$ dm^{-3} = 0.0, Al saturation (%) = 0.0; Base saturation (%) = 58.2; MO (%) = 2.2, P - Mehlich mg dm^{-3} = 24.8; K mg dm^{-3} = 200.

The water seeded seeding system was used with flood depths formed 20 days before seeding. For each flood depth, the desired height was identified to maintain uniformity. An amount of 40 seeds per tray was used, corresponding to 310 seeds m^{-2} for both genotypes. For pre-germination, the seeds were soaked in water for a period of 36 h. After this period they were removed from the water and placed in the shade in a protected environment for a period of 36 h until the primary root and coleoptile reached 2 to 3 mm (Sosbai, 2010). After this period, the seeds were seeded manually.

Air, water, soil temperatures and light intensity

The experiment was conducted at air temperatures of 17°C (day and night) and 20°C (day and night) up to 25 days after seeding (DAS), when the experiment was terminated. A 12:12 photoperiod was utilized with a light intensity of 325 µ mol s^1m^2. During this period, temperature of the water near the soil surface was recorded for each flood depth and soil temperature was recorded at a depth of 1 to 2 cm. Water and soil temperatures were monitored with 107-L34-PT sensors, and the data were recorded in a CR1000 data logger.

Growth parameters

Shoot length was measured at 25 DAS (V3 stage). For this evaluation, three plants per tray were collected, and shoot length was measured with a graduated scale. The mean value was obtained by summing the values of the three plants measured and then dividing the sum by the number of plants. In the same period, an evaluation was made of the dry weight of shoots and roots by drying them in a forced air heating system at 65 °C until constant weight.

Nitrogen concentration (N) and N accumulated per plant

To determine the concentration of N and N accumulated plant^{-1}, after the determination of the dry mass of the shoots harvested at 25 DAS, N was determined in these plants with a FLASH 2000 (NCS) autoanalyzer.

Determination of hydrogen peroxide

The concentration of hydrogen peroxide (H_2O_2) was determined according to Loreto and Velikova (2001): 0.05 g fresh matter (roots and shoots) were homogenized in 2 mL of 0.1% trichloroacetic acid. The homogenate was centrifuged at 12,000 g for 15 min. Then, 0.5 ml of the supernatant was added to 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1M KI. The H_2O_2 concentration of the supernatant was measured by comparing its absorbance at 390 nm using a standard calibration curve (Loreto and Velikova, 2001).

Determination of chlorophyll and carotenoids

The concentrations of carotenoids and chlorophyll were determined by the method of Hiscox and Israelstam (1979) and estimated with Lichtenthaler's method (Lichtenthaler, 1987), using 0.1 g of frozen leaves. The leaves were incubated at 65 °C in dimethyl sulfoxide (DMSO) until the tissues were completely bleached. The absorbance of the solution was then measured at 470 nm, 645 and 663 to determine the concentration of carotenoids and chlorophylls.

Estimation of lipid peroxidation

The level of lipid peroxidation products was estimated according to the method of El-Moshaty et al. (1993), by determining the concentration of malondialdehyde (MDA) as the product of lipid peroxidation by reaction with thiobarbituric acid. The mixture was heated at 95 °C for 40 min and then cooled on ice for 15 min. After centrifugation at 5,000 g for 15 min at 4°C, the absorbance of the supernatant was determined at 532 nm. A correction for non-specific turbidity was made by subtracting the absorbance value obtained at 600 nm. Lipid peroxidation was expressed as nmoles of MDA (mg fresh mass^{-1}).

Enzyme activities

One gram of frozen tissue homogenized in 3 ml of 0.05 M sodium phosphate buffer (pH 7.8) including 1 mM EDTA and 2% (w/v) PVP was used for each assay (Zhu et al. 2004). The supernatant was used for the assays of Catalase (CAT), Ascorbate peroxidase (APX) and Superoxide dismutase (SOD). The CAT activity was assayed following the modified Aebi (1984) method. The APX activity was measured according to Zhu et al. (2004) and SOD activity was assayed according to Misra and Fridovich (1972).

Statistical analysis

The measured outcomes were tested by the assumptions of the mathematical model (normality and homogeneity of variance); the variable root dry mass at 25 DAS was transformed by the Box-Cox transformation. The values given are untransformed. The analysis of variance of the experimental data was performed using the F-test. The means of the qualitative factors, when significant, were compared by Tukey's test at 5% probability. The quantitative factor, when significant, was subjected to polynomial regression analysis, by testing the linear and quadratic models. The level of significance was 5% probability of error.

Conclusion

Air temperature and flood depth affect nitrogen absorption, dry matter accumulation and oxidative stress in rice seedlings in the water seeded system, with differences between cultivars. Fewer stresses are observed in rice plants subjected to a flood depth of 1 cm at 20 °C with IRGA 425 being more tolerant to greater flood depth when compared to Epagri 114.

Acknoweldegements

The authors gratefully acknowledge the financial support of Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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