PHYSIOLOGICAL AND BIOCHEMICAL PERFORMANCE OF HYDRIC STRESS-PRODUCED RICE SEEDS

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INTRODUCTION

Rice is drought-sensitive mainly on flowering and grain filling periods, in which damages can be irreversible resulting on big production lost. Seeds are also highly affected to hydric stress, both on formation and culture initial development. Fields with drought incidence produces seeds with lower quality and quantity, once water deficit have direct effect on reserve accumulation and no pollination process.

Those damages caused on culture are effect from increase on reactive oxygen species (ROS) on plant, resulting on disbalance on metabolic activities and the oxidative stress. Research has shown that, due increase on water stress, plant tends to increase ROS production due changes on metabolism caused by water restriction. However, those substances are natural for any aerobic organism and have many metabolic roles (SUZUKI *et al.*, 2012). Antioxidant-complex enzymes are responsible to keep balance and avoid those molecules oxidative activity, in general, those enzymes can transform free radicals on non-toxic products.

The antioxidant complex is composed mainly by the enzymes superoxide dismutase (SOD) and peroxidases as ascorbate peroxidase (APX). SOD is the first defense against ROS, by dismutation of superoxide radical on hydrogen peroxide, which is a less toxic molecule. APX enzymes are more efficiency on ROS's cleaning, with high affinity for the molecules on many cell compartments.

This research was carried aiming to evaluate enzymes from antioxidant complex on relation to the response to hydric stress on reproductive phase on upland rice genotypes.

MATERIAL AND METHODS

It´s used seeds from upland rice breeding program from the agreement between Federal University of Lavras, EPAMIG, and EMBRAPA Rice and Bean. We used three lineages being BRSMG-Relâmpago as the hydric stress tolerant (witness) (DE CASTRO *et al.*, 2014) and lineages

CMG2172 and CMG2093. We used duplicate for each genotype, with 25 seeds each, placed on oven with forced air circulation at 105°C for 24h.

Biochemical analyses were carried by using dried seeds and seedlings from emergency tests. Those were kept on deep-freezer (-80°C) until tests procedures according to following method. We measured superoxide dismutase (EC 1.15.1.1) and peroxidase (EC. 1.11.1.7) through electrophoresis technique. Enzyme extracts were obtained by homogenizing 100mg macerated samples on 250µL buffer {0.2M tris HCl pH 8.0; 0.1% beta-mercaptoethanol). Solution was homogenized on vortex and kept for 12 hours on fridge, followed by 14,000 rpm centrifugation by 30 minutes at 4°C.

Electrophoresis was carried on discontinuous acrylamide gels at 7.5% (separator) and 4.5 (concentrator). System gel/electrode was Tris-glycine pH 8.9. We applied 60µL extract on gel and electrophoresis was carried at 120V for 5 hours under refrigeration 4°C. For each enzyme, we used three gels, representing biological replicates. Each replicate was used for visual analysis of bands intensity using software ImageJ®, at mm^2 unity.

Superoxide dismutase was determined by ability to inhibits photochemical reduction of nitro blue tetrazolium (NBT), proposed by Giannopolitis and Ries (1977). Samples were added to mix composed by 50 nM potassium phosphate, 13 mM methionine, 0.1 µM EDTA, 75 µM NBT, and 2 µM riboflavin. Samples and witness (mix + water instead of extracts) were kept on 20 W fluorescent light on room temperature for 7 minutes. Absorbance at 560 nm was. Each SOD unity is defined by the enzyme necessary to inhibits 50% of NBT reduction. Ascorbate peroxidase (APX) was determined by reduction of ascorbate absorbance at 290 nm at every 15 seconds during 3 minutes according to Nakano & Asada (1981). For this, 9 µL of extracts (or water on witness) were added to 162µL of reaction buffer [100 mM potassium phosphate pH 7.0 + 0.5mM ascorbic acid] previously heated at 30°C. On the mix sample+reaction buffer was added 9 µL hydrogen peroxide (2 mM). Being the absorbance measured on ELISA equipment**.**

RESULTS AND DISCUSSION

Regarding SOD activity (Figure 1), we observed that the lower concentrations of this enzyme can be found on seeds being CMG2172, the cultivar with the lower values for both conditions of field production. On ideal cultivation conditions, as 70% field capacity, produced seedlings had similar results for both seeds from irrigated or not fields. CMG2093 had superior activity on both comparatives regarding the contrasting material. Comparing the environments, we could observe

a decrease on activity on both lineages as could be seen on seeds form fields without irrigation develops into seedlings with lower SOD activity. For seedlings developed on 10% field capacity, on both condition of environment with irrigation for lineages CMG2172 and CMG2093 were superior than witness, and for environment without irrigation had the witness and genotype CMG2093 with higher concentrations. An interesting factor to evaluate the results on this condition is the difference between conditions of these seed productions, indication that plants on extreme conditions tends to produce less SOD.

We observed a correlation between SOD enzyme activity and genotype ability to develop and establish on water deficit conditions, once in the vigor tests, material with higher values for emergency speed and percentage had higher SOD enzyme activity on either ideal or water restriction conditions. Seedlings with higher SOD activity had higher cell protection, once this enzyme is the first way of control the damages caused by free radicals, it have role to initiate detoxication by reducing superoxide radical on hydrogen peroxide. Thus SOD activity on seedling on water deficit is a positive signal regarding plant ability to tolerate environmental adversities, once on low levels, superoxide radical result on less cell oxidative damages due its lower toxicity if compared to hydrogen peroxide (LI, CHUN RONG *et al.*, 2013).

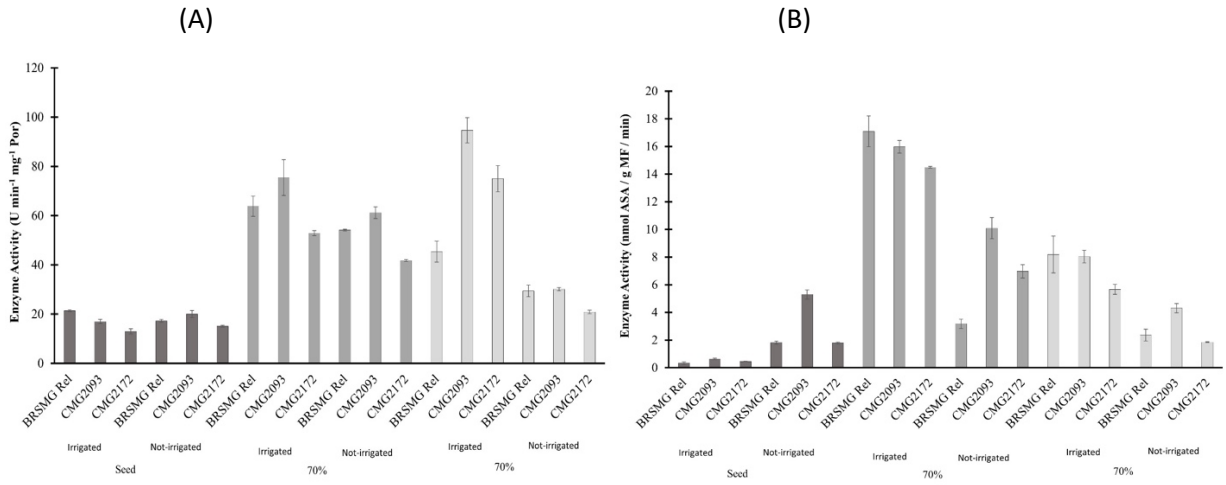

Figure 1. (A) Superoxide dismutase enzyme activity on rice seeds and seedlings produced with and without irrigation on conditions of 70 and 10% field capacity; (B) Ascorbate peroxidase (APX) activity on rice seeds and seedlings from three cultivars on production systems of 70 and 10% field capacity.

The three genotypes, we observed that there is a balance on ascorbate peroxidase (Figure 1) at condition of irrigated fields, which indicates that, on ideal developing conditions, seeds from these lineages have similar response regarding APX enzyme activity. When analyzed genotypes from production fields irrigated or not, a stress incidence could be found once cultivar CMG2093 had higher activity compared to the others, independently of evaluated material. APX have detoxication role which avoid accumulation of hydrogen peroxide on plant system (SINGH *et al.*, 2020). Sohag et al (2020) found on rice seedlings under water deficit with high APX activity had higher tolerance to induced stress. This higher APX activity on seedlings suggests a signal of tolerance to water deficit once H_2O_2 accumulation on different cell compartments creates a stress defense barrier avoiding oxidative damage on fundamental organelles as chloroplasts.

CONCLUSION

Lower damage and higher performance under hydric stress conditions could be observed on CMG2093. Enzyme expression is influenced by hydric stress and each cultivar has different response to hydric stress.

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