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Phenotypic and Enzymatic Analyses of Upland Rice Lines Seeds under No-Tillage

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Abstract

Over 40% of the agricultural area in Brazil corresponds to no-tillage farming. Although it is evident that the importance of obtaining upland rice cultivars is adapted to this cropping system, researches in this sense are still incipient and breeders do not have cultivars available to recommend with enough security. The aim of this study was to evaluate phenotypic characters favorable to the no-tillage system in order to establish the basis for upland rice breeding programs interested in this cropping system. The seeds of twenty elite inbred lines from the Upland Rice Breeding Program from the Brazilian research institutions UFLA, EMBRAPA and EPAMIG were evaluated at the Central Seeds Lab from the Agriculture Department of UFLA following a randomized block design with four replicates. The characters evaluated were seedlings emergence (SE), green matter yield (GM), dry matter yield (DM), photosynthetic activity (PA), and superoxide dismutase activity (SOD). The quality parameters evaluated were obtained by a germination test (GT), germination first count (GFC), emergence test (E), initial stand (IS), and alpha-amylase expression. The lines CMG 2074, CMG 1967, CMG 2017, BRSMG Caravera and CMG 2097 showed higher SOD content in the enzymatic analyses, and consequently higher tolerance to abiotic stresses that could be caused by no-tillage. Considering the evaluation of agronomic traits, the line CMG 1967 showed the best performance in no-tillage soil and, therefore, should receive higher attention in Upland Rice Breeding Programs.

Keywords

***Oriza sativa*, Physiologic Quality, Enzymatic Expression, Cropping System, Vigor**

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1. Introduction

Rice (*Oryza sativa* L.) is among of the most consumed cereals in the world, and is the main source of carbohydrates in Brazil [1]. The rice production in Brazil reached nearly 12,400 millions of tons in 2344 hectares in the 2014/2015 growing season, and the main producer states were Rio Grande do Sul, Santa Catarina and Maranhão, responsible for 70% of the production [2].

Although Brazilian production is mainly of irrigated rice, Brazil has the biggest cultivated area of upland rice in the world, mostly in the states of Mato Grosso, Tocantins and Maranhão [2]. In these regions, rice is cultivated by small farmers without any irrigation, in acid soil, poor of nutrients, low water retention and with irregular rain precipitation [3].

Since the 80s, much of the upland rice has been cultivated in order to start new farmable fields in degraded pastures and deforested areas [2]. Although this strategy has increased the cultivated area, the cultivation of upland rice should not be considered only in this situation, because it does not supply internal demand. In this context, the obtaining of upland rice cultivars adapted to no-tillage system enables rotation with other crops, supplies the rice demand and guarantees another alternative for small farmers.

Besides allowing crop rotation, the usage of no-till system improves soil fertility, erosion protection, infiltration, and water availability, minimizing environmental impacts. However, the successful implementation of this system directly depends on the biomass production in rotation systems, without which the objectives and advantages of this type of cultivation will not be the same [4].

According to the Brazilian Federation of No-Tillage and Irrigation Systems (FEBRAPDP), the area with no-tillage in Brazil has nearly 30 millions of hectares, reinforcing the importance of obtaining upland rice cultivars adapted to this system. Furthermore, rice has a high biomass production that benefits crop rotation. However, the use of upland rice in no-tillage system is not successful. The initial growth and development of the rice seedlings are affected, probably because of the transformation of nitrate, predominant in no-tillage system, in ammonium, which is absorbed and hampers seedling development [5].

Nevertheless, the consolidation of this cropping system is essential to enable rice cultivation in regions where only no-tillage is used, especially because rice production using irrigated systems is reaching the edge. The obtainment of new upland rice cultivars adapted to no-tillage system is the best alternative to supply the increasing demand of rice. The aim of this study was to evaluate upland rice lines for phenotypic characters favorable to the no-tillage system in order to establish the basis for upland rice breeding programs interested in this cropping system.

2. Material and Methods

The seeds of twenty elite inbred lines from the Upland Rice Breeding Program from the Brazilian research institutions UFLA, EMBRAPA and EPAMIG were evaluated at a greenhouse in the Central Seeds Lab from the Agriculture Department of UFLA following a randomized block design with four replicates. Each plot was a 3L plastic pot with four plants sowed in soil from a no-tillage area prepared as recommended for rice crop. The characters evaluated were:

1) Seedling emergence, as the number of days from sowing and the emergence of 50% of the plants from each plot;
2) Green matter yield, as the weight of green matter of one plant from each plot;
3) Dry matter yield, as the weight of the same sample used to determinate the green matter stored in warm house for 72 h in 65˚C and 0% of humidity;
4) Photosynthetic activity, as therelative chlorophyll index (RCI) measured by a chlorophyll meter (SPAD), obtained as the mean of 30 reedsin five leaves;
5) Superoxide dismutase (SOD) activity, obtained by spectrophotometry. Leaves from each genotype were smashed using liquid nitrogen. Then, 0.2 mL of acetone 80% was added to 0.1 g of each sample, mixed for 5 minutes. The samples were centrifuged in 6.000 rpm for 10 minutes and the supernatant discarded. For the enzymes extraction, it was used 0.2 mL of the extraction buffer Tris 40 mM (pH 7.8) containing Manitol 350 mM, EDTA 2 mM and PVP 3%. The samples were centrifuged in 10.000 rpm for 20 minutes in 4˚C. The supernatant were stored in chamber at −20˚C. Then, 0.1 mL of each sample were put in test tubes protected of light with a potassium phosphate buffer (50 mM, pH 7.8, EDTA 0.1 mM, L-methionine 13 mM and NBT 75 μM). The reaction was initiated adding Riboflavin 6 μM and transferring the tubes to a lighted chamber

for 15 minutes. The reeds were made using spectrophotometry (Spectronic 20 Genesys) and the SOD activity was determined calculating the quantity of extract that inhibited 50% of the NBT [6].

Biochemical and physiological analyses were also performed using seeds from each line treated with the commercial fungicide Vitavax-Thiram®, 0.003L by kilogram of seed. The tests performed were:

1) Germination test, using 50 seeds of each inbred line with four replicates, distributed on Germitest® paper moistened with water amount equivalent to 2.5 times the mass of dry substrate, and maintained in germination chamber at 25˚C degrees. Two counts of the number of normal seedlings were performed at the fifth and fourteenth days after sowing, according to recommendation in Brasil [7]. The results were expressed as the average percentage of normal seedlings from the four replicates;
2) Germination first count, performed together with the germination test, considering a seedling as normal when showing 0.5 cm of shoot length and 2.5cm of root length at the fifth day after sowing;
3) Emergence in plat, evaluated sowing four replicates of 50 seeds from each line in a 1m x 4m plat with the lines arranged randomly. Daily assessments were performed from the first day of seedlings emergency until the stabilization of the stand. The results were expressed as the percentage of normal emerged seedlings at the seventh and tenth days after sowing;
4) Initial stand, as the percentage of normal emerged seedlings at the twenty-first day after sowing;
5) Alpha-amylase expression, inferred considering the intensity of bands of electrophoretic analysis in a transilluminator. Two samples of 25 seeds from each line were soaked with water for 72 hours and then smashed using PVP (Polyvinylpolypyrrolidone) and liquid nitrogen. For the enzymes extraction, it was used an extraction buffer (Tris HCl 10.2 M pH8 + 0.1% of β-mercaptoetanol) in the proportion of 250 µL by 100 mg of sample. The samples were homogenized in vortex and maintained in fridge during the night. They were centrifuged in 14.000 rpm for 30 minutes in 4^{O}C degrees, and 60 µL of the sample were added to a polyacrylamide gel (7.5% of separator gel and 4.5% of concentrator gel) to perform electrophoretic analysis (150 Volts for 5 hours) using the gel/electrode system Tris-glycine pH 8.9.

Analyses of variance were performed with the data from each character using the statistical software SISVAR [8], and the means compared according to the Scott-Knott approach [9] with 5% of probability. The evaluation of enzymatic patters were performed according to the intensity if the bands [10].

3. Results and Discussion

Highly coefficients of variation (CV%) were observed for all characters, indicating that the experiments were carried out with high precision [Garcia, 1989]. Significant differences ($p \leq 0.05$) between rice lines were detected in the analyses of variance for seedlings emergency and green matter yield. However, for green matter, only the line CMG1967 showed superiority. For the characters dry matter yield and photosynthetic intensity, the lines did not differ statistically (**Table 1**).

Green matter and dry matter yield are directly correlated to the photosynthetic rate and consequently the production of photoassimilates by plants [11]. Similar to this study, no variability for dry matter yield was found in millet genotypes [12]. However, variability for this character was found in sugar cane [13] and lettuce [14]. For green matter, variability was found between genotypes of lettuce [14], irrigated rice [15] and guava [16].

The speed of seedlings emergency is very important in the context of competition with weeds at the initial crop development, as the higher the speed of emergence, the better the chances of reducing competition effect [17]. Seventeen of the twenty lines had good results for this character. Only the lines CMG 2173, CMG 1097-7 and BRS Caravera showed a bad performance and were considered less vigorous. Variability in the speed of emergency was also found in irrigated rice [18] and sugar cane [19] genotypes.

Photosynthetic activity is measured by the content of chlorophyll in the leaves and consequently is correlated with the content of nitrogen, since this nutrient is part of the chlorophyll molecule. Thereby, the photosynthetic activity can be considered an indirect, instantaneous and non-destructive way to estimate the nutritional state of the genotypes in what regards to the N content, and can be used to indicate the need of nitrogen fertilization in any stage of crop development [20].

Lopes *et al.* [21] and Argenta *et al.* [22] showed positive correlations between the rate of total chlorophyll and the content of N in the leaf ($r = 0.81$ and $r = 0.59$). In this study, all twenty lines showed good estimates of the photosynthetic activity, but they did not differed significantly.

According to Takabe and Yoneyama [23], most of rice genotypes do not have a significant quantity of nitrate,

Table 1. Means of days to seedlings emergence (SE), green matter yield (GM), dry matter yield (DM) and photosynthetic intensity (PI) of the twenty upland rice lines and their coefficient of variation (CV).

Lines	SE (days)*	GM (g)*	DM (g)*	PA (IRC, SPAD)*
BRSMG Curinga	7.0 a	12.57 b	5.46 a	42.82 a
CMG 1967	8.0 a	15.32 a	6.06 a	37.60 a
BRS Esmeralda	8.0 a	10.70 b	4.98 a	44.30 a
CMG 2170	7.0 a	12.13 b	5.26 a	33.52 a
CMG 2173	9.0 b	11.30 b	5.03 a	36.35 a
BRSMG Caçula	7.0 a	14.12 b	5.71 a	35.15 a
CMG 1097-7	9.0 b	13.20 b	5.76 a	39.90 a
CMG 2074	8.0 a	10.95 b	4.98 a	35.25 a
CMG 1698	7.0 a	13.65 b	5.56 a	41.32 a
CMG 2085	8.0 a	9.80 b	4.73 a	33.50 a
BRSMG Caravera	9.0 b	9.15 b	4.46 a	35.75 a
BRSMG Relâmpago	7.0 a	12.53 b	5.11a	31.75 a
CMG 1511	7.0 a	10.72 b	4.86 a	39.30 a
CMG 2089	7.0 a	12.00 b	5.26 a	34.72 a
CMG 1896	7.0 a	10.20 b	4.93 a	39.02 a
CMG 2097	7.0 a	12.97 b	5.46 a	37.25 a
CMG 2017	7.0 a	10.37 b	4.76 a	39.10 a
CMG 1977	7.0 a	10.72 b	4.81 a	34.05 a
CMG 1509	8.0 a	11.22 b	5.03 a	40.02 a
CMG 1987	7.0 a	12.98 b	5.46 a	34.30 a
CV (%)	1.03	14.31	15.98	14.15

*Means followed by the same letter at the column do not differ statistically by the Scott-Knott test at 5% of probability.

but some lines can present quantities that should be considered. In this case, the chlorophyll meter is not useful to indicate the need of nitrogen fertilization, since it does not infer about N in nitrate form.

Considering all previous characters, the line that showed the best performance was CMG 1967, and the lines that showed the worse performance were CMG 1097-7 and CMG 2173.

The lines showed variability in the quantitative analysis of the enzyme superoxide dismutase (SOD). Some lines had a high activity of SOD with the increasing of days after emergency (DAE), and some lines had the opposite behavior (**Figure 2** and **Figure 3**).

To avoid the accumulation of reactive oxygen species (ROS) and protect the cells of oxidative damages, plants have a defense system antioxidant enzymatic and non-enzymatic that allows detoxification. This defense mechanism requires the action of various antioxidant enzymes acting in synchrony, among which we can highlight the superoxide dismutase. SOD activity has been related to the response of plants to abiotic stresses [24]. Since the rice lines in this study were sown in substrate from an area in no-till system, there is a possibility that this cropping system generates some kind of stress to the plant.

SOD was the first enzyme studied as a mechanism of defense against damages caused by ROS in cells [24]. Under the same denomination, there are others that catalyze the dismutation of free radicals of O_2 to H_2O_2 [25].

According to Cardoso [26], SOD enzymes show high level of homology between different plant species. However, the number and type of these enzymes varies between plants. Besides that, there are many environmental and biological factors that lead to the accumulation of superoxide in vegetal tissues [27]. Therefore,

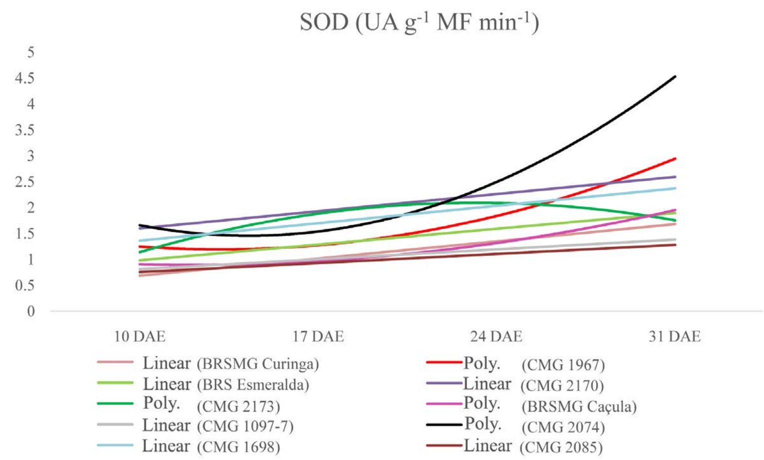

Figure 1. Activity of the enzyme superoxide dismutase (SOD) in leaves of 10 upland rice lines cropped in no-tillage.

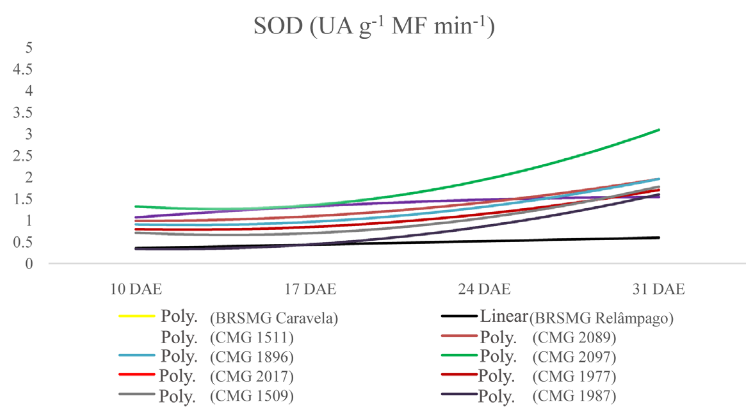

Figure 2. Activity of the enzyme superoxide dismutase (SOD) in leaves of 10 upland rice lines cropped in no-tillage.

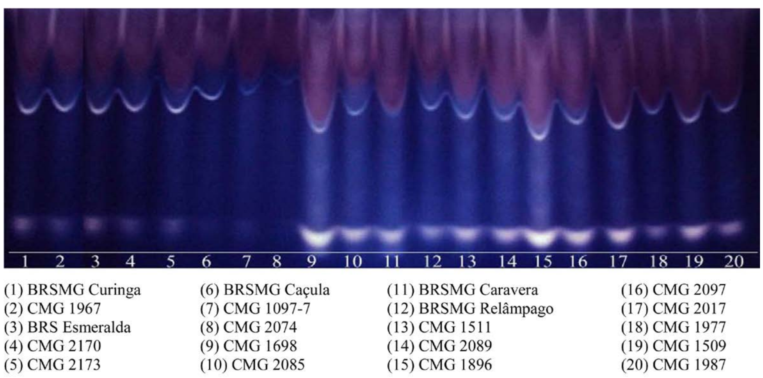

Figure 3. Alpha-amylase expression in seeds of the 20 upland rice lines.

plants can induce SOD synthesis as a defense mechanism [28].

The means of character related to the quality of seeds of the 20 lines are shown in **Table 2**. The lines were statistically different for all characters evaluated.

The germination first count test is based on the affirmation that the genotypes with higher percentage of normal seedling at the twenty-first day after sowing are more vigorous [29]. It is a fast and important test since

Table 2. Percentage of normal seedlings in germination test (GT), germination first count (GFC), initial stand (IS) and emergence (E) in twenty upland rice lines, and their coefficient of variation (CV).

Lines	GFC (%)	GT (%)	IS (%)	E (%)
BRSMG Curinga	66 b	92 a	6 d	89 b
CMG 1967	48 c	76 c	15 c	75 b
BRS Esmeralda	85 a	92 a	75 a	91 a
CMG 2170	72 b	88 a	64 b	82 b
CMG 2173	69 b	77 c	6 d	84 b
BRSMG Caçula	71 b	84 b	17 c	82 b
CMG 1097-7	68 b	96 a	18 c	92 a
CMG 2074	59 c	92 a	14 c	97 a
CMG 1698	72 b	82 b	17 c	93 a
CMG 2085	68 b	90 a	48 b	98 a
BRSMG Caravera	79 a	96 a	23 c	93 a
BRSMG Relâmpago	91 a	95 a	21 c	95 a
CMG 1511	83 a	89 a	26 c	90 a
CMG 2089	54 c	90 a	77 a	95 a
CMG 1896	79 a	90 a	77 a	92 a
CMG 2097	34 d	89 a	59 b	93 a
CMG 2017	79 a	91 a	58 b	96 a
CMG 1977	75 b	91 a	54 b	91 a
CMG 1509	88 a	95 a	74 a	97 a
CMG 1987	49 c	94 a	60 b	91 a
CV (%)	12.46	5.27	20.71	7.27

*Means followed by the same letter at the column do not differ statistically by the Scott-Knott test at 5% of probability.

seedlings emergence speed and uniformity are the most important characters related to the current concept of seeds quality [30].

The lines BRS Esmeralda, BRSMG Caravera, BRSMG Relâmpago, CMG 1511, CMG 1896, CMG 2017 and CMG 1509 had the best performance in the GFC test and, therefore, were considered the most vigorous.

The twenty lines formed three groups with the results of the germination test, discriminating the lines BRSMG Curinga, BRS Esmeralda, CMG 2170, CMG 1097-7, CMG 2074, CMG 2085, BRSMG Caravera, BRSMG Relâmpago, CMG 1511, CMG 2089, CMG 1896, CMG 2097, CMG 2017, CMG 1977, CMG 1509, and CMG 1987 as the ones with best performance, and the lines CMG 1967 and CMG 2173 as the ones with lower quality.

In what regards to the initial stand, the lines formed four groups, discriminating the lines BRS Esmeralda, CMG 2089, CMG 1896 and CMG 1509 as the ones with the best performance. Similar results were found in maize [31] and sorghum [32]. According to Kurachi *et al.* [33], the initial stand can be highly influenced by environmental factors as distribution of the seeds in the sowing line. Due to its plasticity, rice tends to compensate the smaller number of plants in low densities emerging a large number of tillers per plant [34].

The emergency test discriminated the lines BRS Esmeralda, CMG 1097-7, CMG 2074, CMG 1698, CMG 2085, BRSMG Caravera, BRSMG Relâmpago, CMG 1511, CMG 1896, CMG 2097, CMG 2017, CMG 1977, CMG 1509 and CMG 1987 as the ones with high quality.

It is interesting to notice that the lines that showed a gradual accumulation of SOD in their tissues (lines CMG 2074, CMG 1967, CMG 2017, BRSMG Caravera and CMG 2097), also showed high quality in their seeds. These results suggest an association between the content of SOD in vegetal tissues and the tolerance to abiotic stress. Considering all previous tests simultaneously, the lines with high seeds quality were BRS Esmeralda, CMG 1896 and CMG 1509.

The expression of the alpha-amylase enzyme varied between lines but did not show correlation with the seeds quality (**Figure 3**).

Studying the expression of alpha-amylase in seeds of maize lines, Oliveira *et al.* [35] discussed that besides the genes that control the content of amylase in seeds, many other genes may be related to the genetic control of seeds quality, as the genes that participate of the respiration process. This fact may also explain why lines with high quality showed low expression of alpha-amylase in this study.

4. Conclusions

The lines CMG 2074, CMG 1967, CMG 2017, BRSMG Caravera and CMG 2097 showed higher SOD content in the enzymatic analyses, and consequently higher tolerance to abiotic stress.

Considering the evaluation of agronomic traits, the line CMG 1967 showed the best performance in no-tillage system and, therefore, should receive higher attention in Upland Rice Breeding Programs.

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