Morphological and physiological responses to drought stress in a set of Brazilian traditional upland rice varieties in post-anthesis stage

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Abstract

Among the abiotic stresses that can affect the growth and development of the crop, drought is considered one of the main factors that can reduce the global food production. The goal of this study was to analyze the effect of drought stress on several morphological and physiological parameters in ten traditional upland rice varieties. The experimental design set up in a factorial scheme 10 x 2 (varieties x treatments) with four replicates under greenhouse conditions. At the start of the reproductive stage, rice varieties were submitted to control and drought conditions during 30 days and evaluated for physiological and morphological parameters. Rice varieties were considered as tolerant and susceptible to drought stress based on the classification proposed by five different stress tolerance indices (SSI, TOL, SSSI, DYI and DTE). The results suggest that the adopted indices associated with multivariate analysis from the parameters analyzed are efficient to discriminate between tolerant and susceptible rice varieties to the drought stress. Catetão and Plaui were considered as the most tolerant, while Quebra Cachó and Mira were the most susceptible. The analysis of morphological and physiological parameters through multivariate analyses revealed as an important tool to assist breeders in the identification of tolerant and susceptible varieties and to characterize how the varieties alter their metabolism to withstand the drought stress.

Keywords: Harvest index; multivariate analysis; Oryza sativa L.; productivity; tolerance indices.

Abbreviations: Chla_ Chlorophyll a; Chlb_ Chlorophyll b; DAG_ Days After Germination; DTE_ Drought Tolerance Efficiency; DYI_ Drought Yield Index; HI_ Harvest Index; kPa_ Kilo Pascal; MDA_ Malondialdehyde; PCA_ Principal Component Analysis; PC_ Principal Component; RWC_ Relative Water Content; SPAD Index_ Soil and Plant Analyzer Development; SSI_ Stress Susceptibility Index; SSSI_ Modified Severity Stress Susceptibility Index; TOL_ Stress Tolerance Index; UPGMA_ Unweighted Pair Group Method with Arithmetic mean; W.100gr_ Weight of one hundred grains.

Introduction

A wide range of environmental factors negatively influences productivity of agricultural crops. Abiotic stresses are the primary causes of reduced crop growth and productivity, and among them, drought, salinity, temperature, aluminum toxicity, flooding, pollution and radiation are the most frequent factors (Lawlor and Cornic, 2002). Abiotic stresses can reduce crop productivity up to 70% (Boyer, 1982). Drought stress is considered as one of the main abiotic factors that limit global food production (Araus et al., 2002), affecting approximately 64% of all arable land in the world (Cramer et al., 2011). According to Mahajan and Tuteja (2006), abiotic stresses cause the loss of millions of dollars each year due to reduced grain yields, which threaten the sustainability of agriculture and food security.

In the natural environment, plants encounter several unfavorable conditions that interrupt their normal growth and productivity (Umezawa et al., 2006). Among these unfavorable conditions, water stress is the most important limiting factor for crop production and has become a growing and severe problem in many regions of the world (Passioura, 2007). Rice is particularly susceptible to drought stress, and it is estimated that 50% of its global production can be lost due to drought (Bouman et al., 2005). Upland rice varieties are cultivated in Brazil during the rainy season, which occurs from October to December. During this period, the crop is susceptible to times of water shortage that can significantly reduce the productivity (Rabello et al., 2008). Brazil is one of the few countries in the world, where
rainfed rice is important for domestic supply, acting as a price regulator, as well as, providing income alternatives for small farmers (Conab, 2013). Current estimates show that production of one kilogram of rice may require up to 3,000 liters of water, which is approximately 2-3 times greater than other cereal crops (BoUMAN et al., 2007). Therefore, the development of rice cultivars tolerant to water stress is one of the main challenges of the world agricultural research, since rice plants are considered, evolutionarily and semi-aquatic (Wassmann et al., 2009).

Upland rice varieties have been widely used in breeding programs around the world. These varieties are traditionally grown in environments subject to abiotic stress and may have “rare” genes and alleles that can be easily transferred to other varieties by genetic transformation. This knowledge can contribute to increase the tolerance of plants during water shortages (UGA et al., 2013; SILVEIRA et al., 2015; SINGH et al., 2016).

The selection of plants tolerant to water stress can be performed through the evaluation of morphological and physiological parameters associated with the study of tolerance indices. The analysis of these indices may be performed using different equations. However, the ideal index should not prioritize the production in only one treatment (DarvISHZADEH et al., 2010). Productivity indices are widely used as indicators capable of identifying tolerant varieties to drought stress in several crops, such as rice (Kumar et al., 2014b), beans (Ramirez-Vallejo and Kelly, 1998), barley (Zare, 2012) and wheat (Guendouz et al., 2012). In our work, we have used an association of five different indices, which have been extensively studied to select plants tolerant to the drought stress (Fisher and Maurer, 1978; RAMAN et al., 2012).

Kumar et al. (2014b) analyzed a set of rice genotypes under reproductive stage submitted to drought stress and controlled conditions during two years. The analysis using different indices allowed identifying several genotypes tolerant to the drought stress. These drought tolerant genotypes also showed superior performance with respect to grain yield and desired physiological and biochemical traits such as relative water content, chlorophyll content, proline content, stomatal conductance and photosynthetic rate. Evaluation of physiological parameters has also become an important ally in the selection of varieties tolerant to the drought stress (Cha-Um et al., 2010). For this reason, the present work aimed to evaluate morphological and physiological responses of ten upland rice varieties submitted to control and drought treatment in post-anthesis stage.

Results

Productivity reduced in all varieties submitted to drought stress (Fig 1). However, varieties with higher tolerance showed lower reduction than others that were more susceptible. Catetão and Piauí varieties showed a reduction of only 25.4% and 27.9%, respectively, whereas Mira and Quebra Cacho varieties showed the largest reduction, 62.9% and 63.2%, respectively (Fig 1 and Table 51). Drought tolerance was estimated based on five different drought tolerance indices (Table 1). Catetão, Manteiga and Piauí showed the lowest SSI, TOL, SSSI and DYI values. On the other hand, Quebra Cacho and Mira varieties showed the highest SSI, TOL, SSSI and DYI indices. DTE shows the highest values for the first group mentioned, while for the second group an opposite result was observed. A positive correlation was observed between SSI, TOL, SSSI and DYI indices (Table 2). Based on these indices, low values indicate the most tolerant varieties, while high values indicate the most susceptible varieties to the drought stress. Opposite result may be observed for the DTE index, which correlates negatively with the other indices (Table 2). Catetão and Piauí were selected as the most tolerant, while Quebra Cacho and Mira as the most susceptible (Table 1).

The morphological and physiological parameters analyzed were grouped by the UPGMA hierarchical clustering method. The varieties were grouped into five large groups (Fig 2). Varieties that remained close in both treatments did not suffer major alterations in their parameters analyzed, while varieties that significantly altered their variables showed a tendency to separate the treatments in different clusters. Drought tolerant varieties were in group I and II, ie: Bico Ganga, Catetão and Piauí. Varieties that maintained the treatments control and drought stress closer, such as Bico Ganga and Catetão showed strong stability in the parameters analyzed during drought stress. The susceptible varieties were in groups III and IV. The most susceptible varieties were in group V, opposed to the tolerant ones, such as Manteiga, Três Meses and Quebra Cacho.

The varieties were submitted to PCA analysis (Fig 3). The morphological parameters analyzed explained 70% of the total variability in the first two components, where 45% is explained only by PC1 due to the contributions of all morphological parameters, except for the spikelet sterility variable (Fig 3A). Varieties were almost exclusively grouped between control treatments (odd numbers) and drought stress (even numbers).

Under drought stress treatment, Catetão and Piauí varieties were grouped with the control treatment, confirming the results of the clusters (Fig 2 and 3A). The percentage of spikelet sterility was responsible for the separation between varieties submitted to the control and drought stress, especially for Quebra Cacho and Mira varieties. The plant size, leaf, stem and roots weight correlated positively with each other, and are strongly associated with Piauí variety in both control and drought treatment. These characteristics were also associated with Mira and Palha Murcha during control condition. However, weight of 100 grains, productivity, number of panicles, number of tillers and harvest index were the characteristics strongly associated to Catetão variety in both control and drought. Similar result was observed to Quebra Cacho under control condition.

The physiological parameters analyzed explains 80% of the total variability in the first two components (Fig 3B), 42% of which is explained by PC1 due to the contributions of carotenoids, chlorophyll a and b in the varieties submitted to the drought stress, such as Prata Ligeiro, Sempre Verde and Catetão. On the other hand, PC2 explains 38% of the variability, by the positive contributions of RWC and SPAD index associated with the varieties in the control group. PC2 had also the negative contributions of MDA and EE variables associated with the most susceptible varieties, such as Quebra Cacho, Manteiga and Três Meses.
Table 1. Stress tolerance indices analyzed in ten rice varieties submitted to control and drought treatment during the reproductive stage under greenhouse conditions.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>SSI</th>
<th>TOL</th>
<th>SSSI</th>
<th>DYI</th>
<th>DTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bico Ganga</td>
<td>0.92C</td>
<td>0.99C</td>
<td>-0.03C</td>
<td>0.95B</td>
<td>60.76C</td>
</tr>
<tr>
<td>Catetão</td>
<td>0.50E</td>
<td>0.62D</td>
<td>-0.21D</td>
<td>0.73C</td>
<td>78.67A</td>
</tr>
<tr>
<td>Manteiga</td>
<td>0.59E</td>
<td>0.42D</td>
<td>-0.18D</td>
<td>0.77C</td>
<td>74.89A</td>
</tr>
<tr>
<td>Mira</td>
<td>1.54A</td>
<td>1.68B</td>
<td>0.23A</td>
<td>1.68A</td>
<td>34.20E</td>
</tr>
<tr>
<td>Palha Murcha</td>
<td>1.02C</td>
<td>1.43B</td>
<td>0.01C</td>
<td>1.03B</td>
<td>56.47C</td>
</tr>
<tr>
<td>Piauí</td>
<td>0.73D</td>
<td>0.81C</td>
<td>-0.12D</td>
<td>0.84C</td>
<td>68.79B</td>
</tr>
<tr>
<td>Prata Ligeiro</td>
<td>1.15B</td>
<td>1.34B</td>
<td>0.06B</td>
<td>1.13B</td>
<td>51.05D</td>
</tr>
<tr>
<td>Quebra cacho</td>
<td>1.43A</td>
<td>2.46A</td>
<td>0.18A</td>
<td>1.49A</td>
<td>38.98E</td>
</tr>
<tr>
<td>Sempre Verde</td>
<td>0.78D</td>
<td>0.66D</td>
<td>-0.10C</td>
<td>0.86C</td>
<td>66.83B</td>
</tr>
<tr>
<td>Três Meses</td>
<td>0.95C</td>
<td>1.04C</td>
<td>-0.02C</td>
<td>0.96B</td>
<td>59.59C</td>
</tr>
</tbody>
</table>

C.V% |
13.8 | 16.2 | 3.0 | 10.6 | 9.6 |

*aMeans followed by the same capital letter in the column do not differ statistically by the Tukey’s test at p ≤ 0.05 of significance.

Fig 1. Productivity (ton. ha\(^{-1}\)) of ten upland rice varieties submitted to control and drought treatment during the reproductive stage under greenhouse conditions. Same letters do not differ statistically by the Tukey’s test at p ≤ 0.05 of significance. Standard errors represent averages of 4 replicates per treatment.

Table 2. Correlation analysis among the different tolerance indices applied in ten rice varieties submitted to control and drought treatment during the reproductive stage under greenhouse conditions.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SSI</th>
<th>TOL</th>
<th>SSSI</th>
<th>DYI</th>
<th>DTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSI</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOL</td>
<td>0.89</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSSI</td>
<td>1.00</td>
<td>0.81</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DYI</td>
<td>0.96</td>
<td>0.86</td>
<td>0.92</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DTE</td>
<td>-1.00</td>
<td>-0.89</td>
<td>-1.00</td>
<td>-0.96</td>
<td>1</td>
</tr>
</tbody>
</table>

Values in bold are significant by the Tukey’s test at p ≤ 0.01.
Fig 2. Dendrogram of genetic dissimilarities based on morphological and physiological parameters analyzed in the ten upland rice varieties submitted to control and drought treatment during the reproductive stage under greenhouse conditions. The varieties were grouped in five principal clusters. Varieties that remained with both treatments close were considered drought tolerant, while varieties that separate their treatments in distant clusters were considered drought susceptible.

Fig 3. Principal components analysis (PCA) based on morphological parameters, yield components (A) and physiological parameters (B) in ten upland rice varieties submitted to control and drought treatment during the reproductive stage. 1, 2 – Bico Ganga control/drought; 3, 4 – Catetão control/drought; 5, 6 – Manteiga control/drought; 7, 8 – Mira control/drought; 9, 10 – Palha Murcha control/drought; 11, 12 – Piauí control/drought; 13, 14 – Prata Ligeiro control/drought; 15, 16 – Quebra Cacho control/drought; 17, 18 – Sempre Verde control/drought and 19, 20 – Três Meses control/drought. PCA analysis explains most of the total variability in the first two components. The analysis allowed the separation between varieties that were grouped, almost exclusively, in control (odd numbers) and drought stress (even numbers) according to the main parameters analyzed.
The PCA analysis for the physiological parameters also allowed the separation between varieties that were grouped, almost exclusively, in control (odd numbers) and drought stress (even numbers). The varieties in the control treatment were grouped according to the values of RWC and SPAD index, while the varieties in the drought treatment were grouped according to the values of carotenoids, chlorophyll a/b, MDA and EE.

Discussion

The selection of superior varieties for environments subject to stress may be assessed by the analysis of phenotypic characteristics. In our study, the varieties showed marked reduction of shoot and roots weight under drought stress, which affected productivity. Similar results were obtained for Raman et al. (2012) and Usman et al. (2013) in rice varieties submitted to drought stress. However, some varieties presented mechanisms that allow them to withstand the stress period with lower biomass decrease and productivity (Fig 1 and Table S1).

The production of grains has been used as the main component for the selection of plants tolerant to drought stress (Jnandabhiram et al., 2012). In rice, moderate drought stress has been largely characterized by a reduction of 31 - 64% of grain yield, when compared to irrigated condition (Kumar et al., 2008). This result underscores the stability of Catetão and Piauí varieties, which presented a reduction below this range for moderate drought stress (26.4% and 27.9%, respectively). Mira and Quebra Cacho varieties had a reduction of 62.9% and 63.2%, respectively, compatible with the upper limit for moderate stress (64%). These varieties may be considered highly sensitive to drought stress under this condition.

Stress tolerance indices have often been adopted as a tool that assists the selection of drought tolerant genotypes in several agricultural crops (Kumar et al., 2014a). The low values of SSI, TOL, SSSI, DVI and the high values of DTE indicate genotypes with high tolerance based on productivity under drought stress. These indices are more useful to identify genotypes that have a better performance in this condition (Raman et al., 2012). A positive correlation between these indices was observed, except for the DTE index, which correlated negatively with the others. The result shows the possibility of using any of these indices for the selection of genotypes tolerant to the drought stress (Table 2).

Choukan et al. (2006) and Terra et al. (2010) showed that SSI values lower than 1.0 indicate varieties with high stress tolerance. Kumar et al. (2014b) classified the results obtained into four categories: highly tolerant (SSI < 0.50), tolerant (SSI: 0.51-0.75), moderate tolerant (SSI>1.0) and susceptible (SSI > 1.0). According to this scale, the most tolerant varieties were Catetão (0.50) and Piauí (0.73), while the most sensitive were Mira (1.54) and Quebra Cacho (1.43) (Table 1).

The SSI index has been widely used for the selection of drought tolerant genotypes (Guendouz et al., 2012, Raman et al., 2012, Naghavi et al., 2013, Kumar et al., 2014b). Catetão, Manteiga, Piauí and Sempre Verde varieties showed the lowest values of SSI (0.50, 0.59, 0.73 and 0.78, respectively) and the highest values of DTE, indicating that these varieties developed mechanisms to tolerate water stress and ensure the production of grains even in this limiting condition. Manteiga variety presented low productivity in the control condition, which contributed to the low SSI (0.59) observed (Table 1 and S1). This result shows that selection based on indices alone can lead to mistaken choices of supposedly tolerant varieties, which does not occur when the multivariate analysis is associated with the use of the indices. A multivariate analysis considering a set of morphological and physiological parameters was performed (Fig 2). This analysis has been routinely applied to discriminate tolerant and susceptible genotypes to the drought stress (Darvishzadeh et al., 2010; Abdi et al., 2013). In our study, this tool helped to identify Bico Ganga, Catetão and Piauí as the tolerant ones, while Manteiga, Três Meses, Mira and Quebra Cacho as the susceptible ones. The PCA analysis showed that productivity and spikelet sterility index are the main characteristics responsible for the discrimination between tolerant and susceptible varieties (Fig 3A). According to Castillo et al. (2006) and Mostajeran and Rahimi-Eichi (2009) drought stress can reduce translocation of sugars to the grain, which reduces yield, weight and increases the percentage of unfilled grains. The RWC showed negative correlation with MDA and EE (Fig 3B and S1). This result agrees with the literature for different plant species, in which plants with better water status have a lower rate of membrane damage and lipid peroxidation (Basu et al., 2010; Mizraee et al., 2013). Liu et al. (2008) observed that tolerant varieties tend to have a better water status that directly influences grain filling, spikelet sterility and productivity. This result was observed in our study for Catetão and Piauí varieties (Fig 1 and 3). The SPAD index, carotenoids, chlorophyll a and b significantly correlated with the drought stress tolerance. The SPAD index correlated strongly and negatively with MDA and EE (Fig 3B), chlorophyll a and b are stable in Catetão, while strongly reduce in Mira under drought stress (Fig S2). Mostajeran and Rahimi-Eichi (2009), showed that tolerant varieties to the drought stress are able to retain green colors longer than susceptible varieties under drought stress. In addition, tolerant varieties can maintain and/or stimulate the biosynthesis of chlorophylls and carotenoids, which are fundamental for the correct modeling, assembly and insertion of photosynthetic proteins in the thylakoid membranes (Horn and Paulsen, 2002). Analysis based on the morphological and physiological parameters associated with stress tolerance indices allowed identifying Catetão and Piauí varieties as the most tolerant ones. These varieties present different strategies to withstand with the drought stress. In one hand, Catetão variety invests more in the reproduction (tilering, number of panicles, productivity and HI), showing more control of the water status and pigments. On the other hand, Piauí invests, mainly, in growth (plant size, leaves, stems and roots). Quebra Cacho and Mira were considered as the most susceptible to the drought stress due to the negative effects on the yield components and greater susceptibility to membrane damage and electrolyte leakage.

Materials and Methods

Plant materials

The varieties Prata Ligeiro, Três Meses, Catetão, Manteiga, Palha Murcha, Quebra Cacho, Piauí, Sempre Verde, Bico
Ganga and Mira, traditionally cultivated in upland conditions, were used in this study.

**Experimental conditions**

The experiment was conducted under greenhouse conditions. Rice seeds were initially washed in 2% of sodium hypochlorite solution for 10 minutes. The plants were germinated in growth chamber (500 µmolons photons m⁻² s⁻¹; photoperiod 12 h and 70% humidity). 10 days after germination (DAG), the seedlings were transferred to greenhouse and conditioned in tubes (20 cm of diameter × 1.0 m of height) containing soil. 40 kg ha⁻¹ of N as urea and 60 kg ha⁻¹ of K₂O as K₂SO₄ was applied. The experimental design was completely randomized, in a 10 × 2 factorial scheme (varieties × treatments) with four replicates. The treatments were applied at the reproductive stage when more than half of the plants of each variety reached anthesis.

**Treatments**

The water treatments were: control, where the moisture content was maintained close to the field capacity (20 kPa) and moderate water stress, which consisted in maintaining the potential between 50-60 kPa. Control of soil water tension was performed by tensiometers installed in the columns at a depth of 30 cm. Irrigation was performed daily by applying a low dose of water that allowed the maintenance of tension in the desired range. The plants remained in the treatment conditions during 30 days until harvest.

**Morphological parameters**

Plants were segmented into leaf, panicle, stem and roots at harvest. The dry weight was obtained after three days with forced air circulation at 60°C (Table S2). Productivity, spikelet sterility index, weight of 100 grains and harvest index were obtained (Table S1). The number of tillers and panicles were counted and the plant size was determined from the average of the two largest tillers of each plant (Table S3).

**Tolerance indices to drought stress**

Drought tolerance was estimated based on the calculation of five different drought stress tolerance indices. Stress Susceptibility Index (SSI), Stress Tolerance Index (TOL), Modified Severity Stress Susceptibility Index (SSSI), Drought Yield Index (DYI), and Drought Tolerance Efficiency (DTE) (Fischer and Maurer, 1978; Fischer and Wood, 1981; Rosielle and Hamblin, 1981; Singh et al., 2011; Raman et al., 2012.).

- **SSI** = (1 - (Yₛ/Yₚ)) / (1 - (Ŷₛ/Ŷₚ)) Eq.1
- **TOL** = Yₚ – Yₛ Eq.2
- **SSSI** = (1 - (YS/Yp)) - (1 - (Ŷₛ/Ŷₚ)) Eq.3
- **DYI** = (YS/Yp)/(Ŷₛ/Ŷₚ) Eq.4
- **DTE** = (Ŷₛ / Yₚ) × 100 Eq.5

Yₚ = productivity in the control, Yₛ = productivity in the drought, Ŷₛ = mean of all varieties in the drought treatment, Eq. = equation.

**Physiological parameters**

The SPAD index was determined over time at 1, 5, 10, 20 and 30 days after drought treatment (Table S4). At harvest, leaves +2 were collected from each variety and the physiological analyses of relative water content (RWC, %) (Weatherley, 1950), membrane stability (Bajji et al., 2002) and lipid peroxidation (Heath and Packer, 1968) were determined. Total carotenoids and chlorophylls were determined according to ARNON (1949).

**Statistical analysis**

The results were submitted to analysis of variance and the means were compared by the Tukey’s test. The morphological and physiological parameters were submitted to multivariate analysis through the program R, version 2.14.1. For cluster analysis, the mahalanobis dissimilarity clustering method was used. The consistency test of the clustering pattern was performed using the co-phenotype correlation coefficient (CCC) (Sokal and Rohlf, 1962) and Mantel’s test. The cut-off point in the dendrogram was estimated according to kelley et al. (1996). The ordering method used was principal component analysis (PCA).

**Conclusion**

The analysis of the stress tolerance indices associated to the multivariate analysis allowed discrimination of tolerant and susceptible varieties. In addition, it allowed the identification of the main morphological and physiological parameters involved in the tolerance or susceptibility to the drought stress. The study shed light on the main morphological and physiological parameters that should be considered for the selection of superior genotypes to environments subject to water deficit.

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