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EVALUATION OF INDUCED WATER STRESS RESPONSES IN COMMERCIAL RICE (ORYZA SATIVA L.) GENOTYPES IN THE DOMINICAN REPUBLIC

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ABSTRACT

Due to the negative effects of climatic change on agricultural production, it is becoming more necessary to determine crops with tolerance to adverse environmental conditions such as drought. In order to determine rice (Oryza sativa L.) genotypes with potential tolerance to water stress, the physiological and biochemical responses of commercial varieties grown in the Dominican Republic to induced water stress were analyzed; this by utilizing polyethylene glycol 8000 (PEG-8000) as stress inductor. Said substance tend to cause osmotic stress thanks to its viscosity under certain temperatures, restricting the transport of water through the plant membranes leading to water deficit. According to preliminary results, the varieties Jaragua and Quisqueya performed relevant physiological responses by having larger seedlings and roots which indicates adaptations for water browsing. On the other hand, the variety Aceituno had surprising biochemical responses with the highest accumulation of chlorophyll b which is attributable to the light conditions that seedlings were submitted, also, as a defense to water stress by carrying the function of replacement molecule for energy transport in the Photosystem II during photosynthesis, because of the dehydration of chlorophyll a. These results suggest the existence of a tolerance index among rice varieties grown in the Dominican Republic.

Keywords: Rice, Water Stress, Drought, Tolerance, Peg-8000.

1. INTRODUCTION

The cultivation of rice (*Oryza sativa* L.), a model organism among cereals and the main staple food in the world's diet, is one of the major approaches in terms of water use due to its semi-aquatic nature. It is an annual grass mainly produced in Asia and between 2016-2017 FAO reported an average production of $5x10^8$ tons and it is expected to increase up to $2x10^9$ tons by 2030. This increment is due to the currently increasing overpopulation even with the current problems of climatic change (Núñez and Hodai, 2021; Panda et al., 2021). It is forecasted that these environmental changes will end in a drought that; undoubtedly, will affect the agricultural production of highly important crops such as rice.

As of December 2022, FAO established that the expected cereal production for the 2022-2023 period could reach $8.5x10^8$ tons (FAO, 2022), which is below of needed to meet the forecasted demand of 2030. Improving plant production methods, including the application of biotechnological techniques to obtain more productive and environmental-stresses-tolerant varieties is necessary. Currently, the responsible consumption of water is one of the objectives of the 2030 Agenda regarding the adequate use of natural resources under the current and severe effects of climatic change on food production and water use (UNDP, 2022). Previous reports indicate that in the case of rice, drought; and its consequent effects (low rainfall, soil erosion, fluctuations in water levels, high temperatures, among others) is the main cause of crop losses and therefore of agro-industrial production (Panda et al., 2021).

Regarding plant physiology, water is the main component of the activity of nucleic acids, metabolism, proteins, and other cellular components (Azcón-Bieto and Talón, 2008). Drought stress in rice cultivars causes severe damage from germination due to the amount of water it needs for its biological cycle. Consequently, inhibiting the water balance, influencing cellular metabolic processes like photosynthesis, membrane transport, and ATP production. Therefore, affecting the subsequent vegetative development and shows morphological aberrances (Panda et al., 2021).

Rainfed rice cultivation has been studied from the point of view of production in areas with subsistence agriculture. It depends on the rain for its development and subsequent yield (which is generally less than irrigated rice) and its sowing is unstable, therefore making it unreliable for commercial production; generating a need for proper research on this topic. In addition, the varieties adapted to irrigation, tend to adapt deficiently to dry farming (Moquete, 2010). Therefore, it is essential to determine tolerant genotypes to this phenomenon and implement them in plant breeding programs. In this sense, the Dominican Republic has, albeit informally, dryland rice fields where the use of water is practically null, providing signals of genotypes of low water consumption for their sustainable and industrialized production. Said that this research aimed to evaluate the potential to tolerate simulated water deficit by using polyethylene glycol (Susilawati et al., 2022; Florido et al., 2018; García et al., 1999) in commercially characterized rice genotypes grown in the Dominican Republic, these were: *Oryza sativa* L. var Juma-67, Quisqueya, Jaragua and Aceituno as they occupy the majority of the cultivated area in the country (Núñez and Hodai, 2021) as a preliminary study for further plant breeding and genotype selection purposes.

2.MATERIALS AND METHODS

2.1.Plant material

Samples harvested and donated between 2018-2019 and fresh seeds collected in 2022 from the Juma Experimental Station of IDIAF, located in Monseñor Nouel province (18° 88” N, 70° 36” W); stored at 3-5°C, were used. Previous to sowing, the seeds were immersed in sodium hypochlorite (NaOCl) 5% g/v mixed with two drops of Tween-20 and shaken for 5 minutes at 120 rpm. Distilled water was used afterward to rinse the seeds twice. The chosen varieties were: *Oryza sativa* L var. Juma-67 (J67), Aceituno (A), Jaragua (J) and Quisqueya (Q)

2.2 Germination at day 3, total germination and mean germination time

Seeds that showed the initial phase of embryo germination (seed opening) were considered on the third day of sowing as the day-to-head for the beginning of the said process. As for total germination, seedlings up to 2 mm in height on the seventh day of sowing were considered germinated. The mean germination time was estimated following previous reports (Diédhiou et al., 2021) with slight variants considering the germination on day 3rd and day 7th.

2.3 Seedling and root length

Seedlings were considered germinated once they showed up to 5 mm on the seventh day of sowing. The average seedling length was measured for each replicate with a ruler. Root length was calculated as explained before by considering the longest root.

2.4 Fresh weight

Fresh weight was calculated as the average biomass per replicate obtained at the end of the experiment.

2.5 Chlorophyll content (Chl)

Seedlings were exposed to light/shade conditions after germination for five days to adapt to light conditions. Two weeks after the stress exposure, the chlorophyll content was examined to verify possible damages to photosynthetic metabolism since it has been demonstrated to improve the understanding of plant defense under drought conditions (Nahakpam, 2017). Around 80 mg of fresh leaf tissue was immersed in 10 mL of 95% ethanol and kept in dark at 25°C for 48 hours (Nahakpam, 2017; Reynoso et al., 2001). The supernatant was utilized to measure the content of pigments using a spectrophotometer (Beckman Coulter DU® 730). Total content was calculated as follows (Wintermas and De Mots, 1965) and each one was expressed as Chl $\mu g \cdot g^{-1}$ of fresh weight:

$$Chl_a = 13.70A_{665} - 5.76A_{649}$$

$$Chl_b = 25.80A_{649} - 7.60A_{665}$$

$$Chl_{a+b} = 1000A_{654} / 39.8$$

2.6 Statistical analyses

A completely randomized block design was applied with genotypes, absence (1) or presence (2) of PEG-8000, and the age of the seeds as young (Y) or old (O). Four blocks were set with replication in time and sixteen treatments with three replicates (Petri dishes) per block; each composed of six individuals completing 72 seeds per treatment. Germination was carried out at 30°C under dark conditions on an incubator (Heratherm IGS180, Thermo Scientific).

Six different responses were classified as physiological or biochemical as follows: Germination at day 3 (Ger3d), Germination (GerT), Mean Germination Time (MGT), Fresh Weight (FW), Seedling Length (SL), and Root Length (RL); on the other hand, Chlorophyll a (Chl_a), Chlorophyll b (Chl_b) and Chlorophyll a+b (Chl_{a+b}). Data were analyzed with the software R v4.2.2 (R Core Team, 2022) by ANOVA and ANCOVA with a verification of the coefficient of variation and Shapiro-Wilk test for normality of residuals to meet the assumption of the analysis, and transformation by Log function when appropriate. Means were compared by Fisher's Least Significant Difference (LSD) test with $p \leq 0.05$. A Pearson's correlation matrix was obtained with the package "agricolae (De Mendiburu and Yaseen, 2020) and the graphics were created with the package “ggplot2” (Wickham, 2016).

3. RESULTS

3.1 Germination at day 3

On the third day of sowing, the germination percentage was calculated to estimate the beginning of germination and consequent developmental stages. J67-2y resulted to be the most tolerant cultivar with 91.7% of germination under stress conditions. J-2o also presented a good performance with 88.9% while "A" in all conditions was reduced to 52.8% of germinated seeds on day three (Figure 1A).

3.2 Germination

The germination did not vary within all treatments which is a good signal for posterior plant yielding since almost all the results were +90%. J67-1y was drastically affected in comparison with the highest value (98.6%) showing 79% of germination, however, this result seemed to be attributable to the germination potential of the harvested seeds but not to the cultivar itself (Figure 1B).

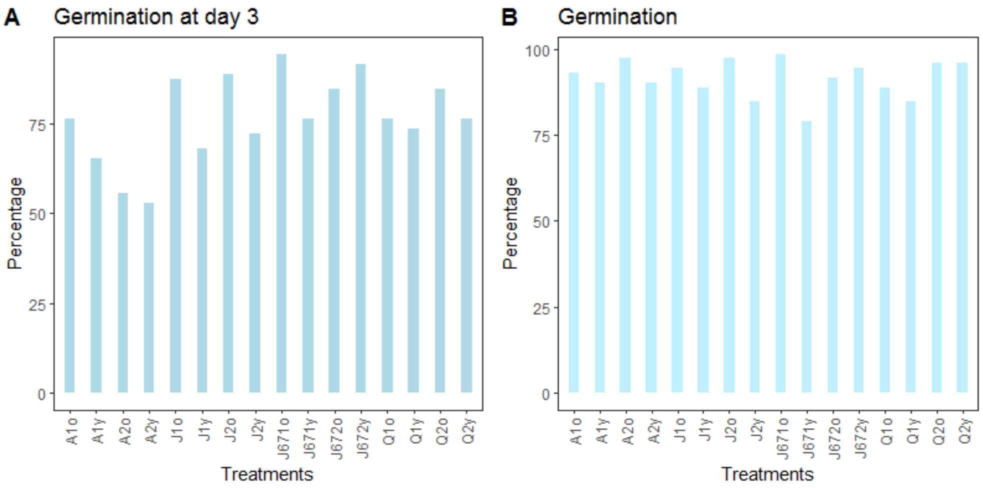

Figure 1. Effects of simulated water stress on germination. A) Germination at day 3; B) Germination; 1 = absence of PEG; 2 = presence of PEG, O = old seeds, Y = young seeds. Two-way ANOVA applied with $P \leq 0.05$ and LSD test for mean comparison.

3.3 Mean germination time

In consonance with the germination at day three, the mean germination time showed positive results for J67-2y with an average time of 3.1 days to fully germinate. Conversely, under the same water stress condition, A-2o and A-2y presented the longest mean germination time of 5 days (Figure 2A). Nevertheless, these results stayed stable for all treatments which imply non-significant variances for practical purposes.

3.4 Fresh weight

In terms of fresh weight, statistical results explained little differences among treatments by separating five groups but not highly different from each other. The highest biomass obtained were A-2O and J67-2O but these weights did not significantly differ from all varieties.

Surprisingly, almost all the young varieties responded with the lowest biomass of 0.09 g, nevertheless, again, it did not differ from all treatments (Figure 2B).

3.5 Seedling growth

After seven days, seedlings showed similar trends for all varieties. J67-1o had highest response according to statistical analyses with 4.96 cm; however, this measurement does not differ greatly from Q-2O and J-2O which showed 4.79 cm under water stress. The stress conditions severely affected J67-2O and A-2O which did not reach more than 3.95 cm. Evaluating the responses of young seeds, it was possible to determine significant effects by age factor for genotype “Q” which under water stress or not, showed a positive adaptation with 5.42 cm long in normal conditions and 5.32 cm (statistically equal) under water stress (Figure 2C).

3.6 Root growth

Root length was affected by stress conditions, and also it was possible to record significant differences by the cultivar. A highlight could be obtained under stress conditions where Q-2O and J-2O reach almost 10 cm, which considerably varies from all varieties, especially under water deficit, decreasing the root length down to less than 7 cm. The young seeds, surprisingly resulted in shorted roots whether water stress was applied or not, nevertheless, the average largest root was obtained from Q-2Y (Figure 2D).

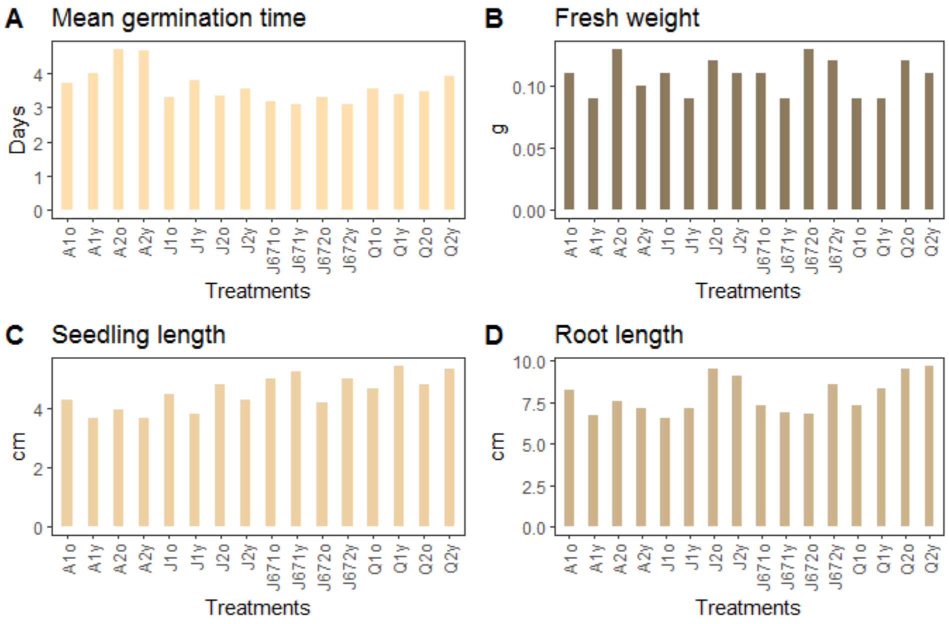

Figure 2. Effects of simulated water stress on seedling development; 1 = absence of PEG; 2 = presence of PEG, O = old seeds, Y = young seeds. Two-way ANOVA applied with *P*≤ 0.05 and LSD test for mean comparison

Table 1. Effects of simulated water stress on physiological development.

Treatments	Ger3d (%)	GerT (%)	MGT (days)	SL (cm)	RL (cm)	FW (g)
Q-1o	76.39 cde	88.89 abc	3.54 cdef	4.63 abcedf	7.31 ef	0.09 e
Q-1y	73.61 def	84.72 bc	3.41 def	5.42 a	8.33 bc	0.09 e
Q-2o	84.72 abcd	95.83 a	3.46 cdef	4.79 abcde	9.48 a	0.12 abc
Q-2y	76.39 bcde	95.83 a	3.92 bc	5.32 ab	9.71 a	0.11 bcd
J67-1o	94.44 a	98.61 a	3.17 f	4.96 abcd	7.30 ef	0.11 bcd
J67-1y	76.39 cde	79.17 c	3.12 f	5.19 abc	6.88 ef	0.09 e
J67-2o	84.72 abcd	91.67 ab	3.30 ef	4.19 defg	7.36 def	0.13 a
J67-2y	91.67 a	94.44 ab	3.11 f	4.97 abcd	8.58 b	0.12 abc
J-1o	87.5 abc	94.44 ab	3.30 ef	4.46 bcdefg	6.50 f	0.11 cd
J-1y	68.06 defg	88.89 abc	3.81 bcd	3.77 fg	7.16 ef	0.09 e
J-2o	88.89 ab	97.22 a	3.33 ef	4.79 abcde	9.49 a	0.12 abc
J-2y	72.22 defg	84.72 bc	3.56 cdef	4.27 cdefg	9.08 ab	0.11 cd
A-1o	76.39 bcde	93.06 ab	3.71 bcde	4.28 cdefg	8.24 bcd	0.11 cd
A-1y	65.28 efg	90.28 ab	4.03 b	3.64 g	6.73 ef	0.09 e
A-2o	55.56 fg	97.22 a	4.73 a	3.95 efg	7.54 cde	0.13 ab
A-2y	52.78 g	90.28 ab	4.66 a	3.66 g	7.13 ef	0.10 de

Same letter within columns did not show significant differences according to LSD $P \leq 0.05$. GerT: germination; Ger3d: germination at day 3; MGT: mean germination time; SL: seedling length; RL: root length; FW: fresh weight

According to ANCOVA analyses, the seedling length and the root length had significant covariance indicating dependences; on the other hand, neither seedling length nor root length correlated with the biomass generated. In terms of correlation, results indicated significant relations among almost all of the physiological variables.

Table 2. Pearson's correlation matrix for physiological responses

	Ger3d	GerT	MGT	SL	RL	FW
Ger3d	1.00					
GerT	0.45	1.00				
MGT	-0.80	0.10	1.00			
SL	0.42	-0.04	-0.55	1.00		
RL	0.16*	0.05*	-0.18*	0.35	1.00	
FW	0.45	0.63	-0.16	0.10	0.27	1.00

*****Non-significant

3.7. Chlorophyll a content

Chl_a content showed a significant decreasing pattern according to the cultivar studied that reaches the peak at J67-1o with 52.2 µg·g^{-1} FW. Statistical results pointed out that the age factor is significantly different as old seeds performed a higher Chl_a accumulation in all cases (Figure 3A).

3.8 Chlorophyll b content

According to the ANOVA analysis, Chl_b presented differences between the treatments resulting from the interaction of age and PEG as well as the cultivar. It was possible to observe the apparent formation of a Gaussian distribution "semi-bell" positively skewed to varieties J and A, highlighting the accumulation of Chl_b in A-1o with 51.18 µg·g^{-1} FW (Figure 3B).

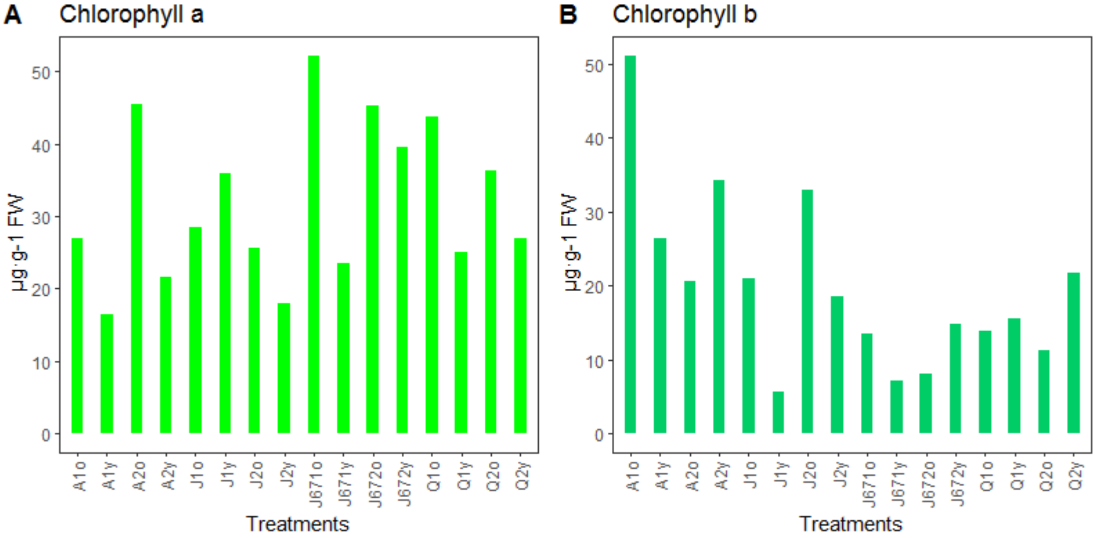

Figure 3. A) Chlorophyll a content; B) Chlorophyll b content; 1 = absence of PEG; 2 = presence of PEG, O = old seeds, Y = young seeds. Two-way ANOVA applied with *P*≤ 0.05 and LSD test for mean comparison

3.9 Chlorophyll a + b content

For Chl_{ab}, ANOVA analysis explained significant interactions between cultivar, PEG and age. In this case, A-2Y and Q-2Y responded with the highest chlorophyll accumulation with 88.02 µg·g^{-1} FW and 82.29 µg·g^{-1} FW respectively. Similar trends were observed in the old seeds of these cultivars. It should be noted; although the water deficit statistically affected this response, the age factor seemed to have more impact. Nevertheless, these responses could also be influenced by environmental conditions (Figure 4).

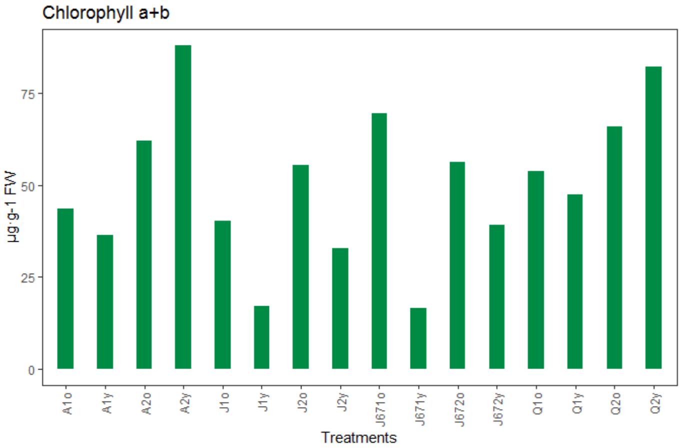

Figure 4. Chlorophyll a+b content; 1 = absence of PEG; 2 = presence of PEG, O = old seeds, Y = young seeds. Two-way ANOVA applied with *P*≤0.05 and LSD test for mean comparison

The ANCOVA analyses revealed a correlation for all biochemical responses recorded in this experiment. Also, significant variances due to age and the interaction of cultivars with PEG-8000 were obtained (Table 3). In this case, Pearson's correlation matrix also showed significant relations (Table 4).

Table 3. Effects of simulated water stress on chlorophyll accumulation.

Treatments	**Chl_a ($\mu g \cdot g^{-1}$)**	**Chl_b ($\mu g \cdot g^{-1}$)**	**Chl_{ab} ($\mu g \cdot g^{-1}$)**
Q-1o	43.73 a	13.82 bcde	53.71 abcd
Q-1y	25.10 abcd	15.60 cde	47.50 bcd
Q-2o	36.3 abc	11.22 cde	65.95 abcd
Q-2y	26.94 abcd	21.64 abc	82.29 ab
J67-1o	52.2 a	13.52 bcde	69.41 abc
J67-1y	23.40 abcd	7.15 de	16.49 e
J67-2o	45.24 ab	8.15 de	56.32 abcd
J67-2y	39.62 abc	14.79 bcde	39.10 cde
J-1o	28.46 abc	21.04 bcde	40.41 bcd
J-1y	35.97 abc	5.70 e	17.20 e
J-2o	25.56 abc	32.91 abcd	55.59 abcd
J-2y	17.98 cd	18.63 abcd	32.82 de
A-1o	26.93 abc	51.18 a	43.66 abcd
A-1y	16.48 d	26.33 abc	36.43 cde
A-2o	45.45 a	20.65 abcd	62.19 abcd
A-2y	21.60 bcd	34.19 ab	88.02 a

Same letter within columns did not show significant differences according to LSD *P*≤0.05. Chl_a: chlorophyll a; Chl_b: chlorophyll b; Chl_{ab}; chlorophyll a+b.

Table 4. Pearson's correlation matrix for chlorophyll accumulation

	Chl_a	Chl_b	Chl_{ab}
Chl_a	1.00		
Chl_b	-0.18*	1.00	
Chl_{ab}	0.28	0.47	1.00

*Non-significant

4.DISCUSSION

Several authors have claimed that drought stress comprises the major cause of damage in cereal crops from abiotic sources, and it is becoming more dangerous due to climatic change (Nahakpam, 2017; Panda et al., 2021; Diédhiou et al., 2021). The principal factor is deficient metabolism due to water limitations; therefore, affections in fluent vegetative development (Azcón-Bieto and Talón, 2008). In our case, the physiology of all varieties seemed to be hurt supporting these statements. However, Q-2 and J-2 presented good seedling and root length which could be attributed to a defense mechanism, especially on root length which was almost 10 cm, tropism that indicates water and nutrient browsing as a survival strategy. This type of response has been reported concerning genotype adaptability to drought stress (Kanjoo et al., 2012; Shereen et al., 2019; Gaballah et al., 2020). Water deficit affects both fresh and dry whole plant's weight (Sikuku et al., 2012; Noryan et al., 2021). Consequently, it is logical to expect that, the larger the plant is, the heavier it would be, but neither varieties nor water deficit appeared to provoke variances among biomass production as minimal significant differences were observed.

Photosynthesis is the fundamental metabolism for growth and development in plants. Some studies have determined that chlorophyll assessments are important to estimate development and posterior yield in rice crops under drought stress (Nahakpam, 2017). Water deficit provoked by drought and heat leads to a loss of water retention which is regulated by stomatal transpiration and therefore, damages the Photosystem II (PSII) efficiency (Restrepo et al., 2014; Piveta et al., 2021). Concerning Chl_a as the main effector of photosynthesis, genotypes did show differences in its accumulation, indicating harmful effects under water deficit as explained above. This pigment is highly sensitive to dehydration, and it has been demonstrated to suffer extreme changes in various crops (Nio et al., 2019) which respond to the behavior observed in this investigation. In terms of the age factor for this metabolite, it is important to explain that the inconsistent pigment accumulation could be due to an environmental factor which influenced the plants' performance and so the amount of healthy tissue able to collect was restricted.

According to ANOVA, the Chl_b and Chl_{a+b} presented statistical variances for age and PEG as well as cultivar interactions. However, it is important to remark on the Chl_b content which drastically increased in J and A varieties, possibly by the effects of the light conditions that the seedlings were submitted to prior to the evaluation of photosynthetic pigments. Because of its role as the second electron transporter in the photosynthesis pathway by passing the electrons, it collects from light to Chl_a so that the plant can perform oxidative phosphorylation. This hypothesis arises according to the exposure by some authors that explain the role of high-intensity light exposure on oxidative stress in rice which increases the cell membrane damage and reduces total chlorophyll content (Nahakpam, 2017; Adhinarayanreddy et al., 2022) as previously detailed in our results. Previous reports established that different concentrations of PEG-8000 did not induce statistical differences among treatments for Chl_b making this not a good parameter for drought tolerance conducted by water deficit (Nio et al., 2019). Commonly, the decrease in Chlorophyll content has been reported under drought stress mainly by damages in the cell membrane due to the presence of saline-type-osmolytes that creates a hindrance over the correct transport of water through plant cells (Ashraf and Bhatti, 2000).

However, a possible reason for the high accumulation of this pigment could rely on the response to light/shade and dark harvesting during growing conditions which affects transcriptional factors responsible for the energy transformation in PSII from Chl_b to Chl_a Eggink et al., 2001; Sakuraba et al., 2012; Niu and Lan, 2022), similarly to the procedures applied in our experiment. In addition, varieties J and A were imported to the Dominican Republic from South America where important agroclimatic conditions certainly vary, especially temperature and solar radiation availability. The adaptation to low light conditions and the high accumulation of Chl_b is common to be a genotype response to stress. It is why is important to keep pursuing studies on stress tolerance and explore new genetic breeding solutions depending on the agroclimatic regions of interaction for crop production. Also, for the sustainability and independence of food security for self-producer countries like the Dominican Republic and keep ahead with the export demand for other countries.

5. CONCLUSION

These preliminary insights into the capability of Dominican rice varieties to potentially tolerate drought conditions setting the water stress as its main factor. Significant variances were observed under stress conditions; which indeed, affected the development at the seedling stage. Quisqueya and Jaragua showed the relevant physiological responses regarding seedling growth, on the other hand, the photosynthetic apparatus was less affected in Aceituno, by increasing the Chl_b metabolism as a defense mechanism to light conditions which magnifies its production.

Although these results give an initial vision of the tolerance index of different rice genotypes commercially cultivated in the Dominican Republic, they also arise the possibility of low tolerance to water deficit since PEG was applied at 10% which implies what could be marked as “low severity”. Because of that, further studies are recommended to verify a tolerance degree considering genetic and molecular responses along with the results here described.

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