Evaluation of the Effect of Drought on the Physiology Activities in Asian Rice (*Oryza sativa* L.)

Modinat A. Adekoya[1], Omolayo J. Ariyo[2], Zaochang Liu[3], Deyan Kong[3], Liguo Zhou[3], Lijun Luo[3]

[1]Department of Plant Science and Biotechnology, Federal University, Oye-Ekiti
[2]Department of Plant Breeding and Seed Technology, Federal University of Agriculture, Abeokuta
[3]Shanghai Agrobiological Gene Centre, Shanghai

*Corresponding author: modinat.adekoya@fuoye.edu.ng

Introduction

Rice is one of the major crops feeding the world population and is most important ingredient in food composition in Asia and Africa. Rice is not only a rich source of carbohydrate and proteins but also provides vitamins, minerals and fibre. It constitutes one of the most important staple foods of over half of the world's population. Globally, it ranks third after wheat and maize in terms of production (Bandyopadhay and Roy, 1992). In 2012/13, about 491.1 million metric tonnes (FAO, 2014) of rice was produced from 158.4 million hectares (Statista, 2014) of land all over the world. Drought is the opposite of flood and it is defined in relation to plant growth or living condition and duration. It is a condition wherein there is continuous dryness or shortage of water to support plant growth and cultivation or living. A drought condition is declared if there is a continuous dryness for more than 15 days. It affects plant cultivation and loss can be up to 90% depending on the extent and duration. Drought stress had become a global concern in food production. It is a limiting factor accounting for most yield loss in the world. Drought is a major threat as weathers are no longer predictable and rainfall lower than normally received due to climate change and global warming (Seck *et al*., 2012; Tao *et al*., 2004). In nature, plant experience multiple or combination of stresses, for example, drought and heat or drought and salinity. In response to heat stress, plants open their stomata to maintain a cooler canopy microclimate through transpiration but under combined heat and drought stress, the sensitive stomata are closed to prevent loss of water, which further increases canopy/tissue temperatures (Rizhsky *et al*., 2002).

Plants are known to develop strategies for coping with stresses when it occur. Stress resistance is based on interaction of the plants with the magnitude and timing of stress - where timing is the stage of plant development when stress occur (Blum, 2011). Exposure of plants to certain environmental stresses can lead to the generation of reactive oxygen species (ROS), including superoxide anion radicals (O_2), hydroxyl radicals ($\cdot OH$), hydrogen peroxide (H_2O_2) and singlet oxygen (O_2^1). Injury caused by ROS, known as oxidative stress, is one of the major damaging factors in plants exposed to environmental stresses such as drought (Price *et al*., 1989). ROS are predominantly generated in the chloroplast by direct transfer of excitation energy from chlorophyll to produce singlet oxygen, or by univalent oxygen reduction at photosystem I, in the Mehler reaction (Foyer *et al*., 1994; Allen, 1995) and to some extent in mitochondria. Chloroplasts are the first targets in plant cells since this is the major site of ROS production. The increased concentration of ROS inhibits the ability to repair damage to photosystem II and inhibits the synthesis of the D1 protein. Stress-enhanced photorespiration and NADPH activity also contributes to the increased H_2O_2 accumulation, which may inactivate enzymes by oxidizing their thiol groups. These cytotoxic active oxygen species, which are also generated during metabolic processes in the mitochondria and peroxisomes, can destroy normal metabolism through oxidative damage of lipids, proteins, and nucleic acids (McCord, 2000). Lipid peroxidation, induced by free radicals, is also important in membrane deterioration (Halliwell, 1987; McCord, 2000). To scavenge ROS, which are accumulated during osmotic stress, the measurement of which includes several physiological activities such as proline content, soluble sugar content, peroxidase or superoxide dismutase activity, chlorophyll content, malondialdehyde content etc. (Luo, 2010), plants have evolved specific defense tactics involving both enzymatic and non-enzymatic antioxidant mechanisms. The objective of this study was to evaluate and compare the scavenging ability of the two varieties of Asian rice to changes in physiological activities due to drought stress.

Materials and Methods

Experimental design: The experiment was laid out in a randomized complete block design with three replicates in the screen-house. Two varieties of rice; Hanyou 8 and Hanyou Xiangqing were used in the experiments. The seeds were sown in plastic box of 68x52x39 cm dimension arranged in three rows in 2012, each representing a water treatment and in four rows in 2013 due to inclusion of an additional water regime. The boxes were filled with soil to brim and compacted to remove air spaces. The soil was later wet to saturation before sowing the seeds. The seeds were sown 20x13.6 cm spacing with a border spacing of 16x6.8cm in 2012 and 13x13 cm spacing with a border spacing of 8x6.5 cm in 2013 thus achieving a seeding rate of 20 seeds per box and one seedling per hill. Three water regime treatments were used in this experiment in 2012, (300, 600 and 900 m^3/mu) while four water regimes were used in 2013 (300, 450, 600 and 900 m^3/mu). The 900 m^3/mu is likened to the paddy flooded field.

Determination of Chlorophyll content of samples: 95% ethanol

Without the veins, 0.05 g leaf sample was accurately weighed in a 50 mL centrifuge tube with cover, placed in a tube rack. 20 mL of 95% ethanol was then added and the rack with the tubes were covered with paper or aluminum foil and kept from light for 24 hours till the leaves turn white. Chlorophyll content was determined at optical density (OD) of 649 nm and 665 nm, using 95% ethanol as control.

Proline content extraction: 0.05 g grinded leaf sample was weighed in 2 mL micro-centrifuge tube and immediately 500 µL 3% of sulfosalicylic acid solution was added, shaken well and placed in the ice box temporarily (spiral vortex was used at low speed rotation to fully mix), and then was put in water bath at 100^0C, for 10 min (accurate timing) (always shaking during the extraction process and avoiding the opening of the centrifugal tube cover so that water will not get into the reaction). After cooling at room temperature, it was centrifuged at 4000 rpm for 5 min. 200 µL of the supernatant was taken, 200 µL glacial acetic acid and 200 µL of 2.5% ninhydrin solution was added. The reaction was placed in water bath at 100^0C heating for 30 min, the solution thus turned red. After cooling, 400 µL toluene was added and the solution mixed thoroughly for 30 s. It was allowed to stand for 2 min and the upper proline red toluene solution was gently taken out into the colorimetric cup for determination, using toluene solution as control. Absorption level was measured at OD value of 520 nm wavelength.

Soluble sugar content: 0.02 g grinded leaf sample was weighed in a 20 mL centrifuge tube, 5 mL distilled water was added and the tube was placed in boiling water bath for 30 min then allowed to cool. 50 uL supernatant was taken into a 1.5 mL centrifuge tube, 150 uL distilled water, 50 µL anthrone reagent and 500 µL sulfuric acid was added and was thoroughly mix. The tube was immediately placed in a boiling water bath (accurate thermal insulation) for 1 min. The solution was allowed to cool to room temperature then absorption level was measured at 620 nm OD using distilled water as control.

Content of soluble protein: 0.05 g grinded leaf sample was weighed in a 2 mL tube, 1 mL 0.9% NaCl was added, put on ice and centrifuged at 4^0C, 8000 rpm for 25 min. 50 µL of the supernatant was taken into a new 2 mL tube, 50 uL distilled water and 500 µL coomassie brilliant blue reagent was added, shake well and placed to rest for 2 min at room temperature. Absorption level was determined at 595 nm, with distilled water as control.

Determination of malondialdehyde (MDA) content: 0.05g grinded leaf sample was weighed into a 2 mL tube, 500 µL 5% trichloroacetic acid (TCA) was added and mixed thoroughly then centrifuge at 300 rpm for 10 min. 200 µL of the supernatant was taken into another 2 mL tube, 200 µL 0.67% thiobarbituric acid (TBA) was added, mixed and put in boiling water bath for 30 min. Solution was allowed to cool to room temperature and then centrifuged at 3000 rpm for 10 min. 250 µL of the supernatant was taken into colorimetric cup and absorption was measured at 450, 532 and 600 nm using distilled water as control.

Peroxidase (POD), total superoxide dismutase (T-SOD) and catalase (CAT) were determined by the method in the protocol for determination by Nanjing assay kit.

Enzyme extraction: 0.05 g grinded leaf sample was weighed, 1 mL normal saline was added, mixed by vortexing for 1 min then centrifuged at 8000 rpm, 4^0C for 25 min. The supernatant was used for the determination of POD, SOD, CAT and soluble protein

Leaf relative water content (LRWC) %

Leaf samples were taken during drought stress occurrence in air-tight polythene bags over ice to stop further physiological activity and immediately taken into the laboratory to take the fresh weight (FW). Each leaf sample harvested was the youngest fully expanded leaf, cut with scissors. The leaf samples were immediately hydrated to full turgidity overnight in the refrigerator, were taken out and dried well to remove any surface moisture with absorbent paper, then immediately weighed to obtain the turgid weight (TW). Samples were then oven dried at 80^0C for 24 h and weighed to determine dry weight (DW). The LRWC is calculated:

$$RWC\ (\%) = [(FW-DW) / (TW-DW)] \times 100$$

Where, *FW*-sample fresh weight, *TW*-sample turgid weight, *DW*-sample dry weight

Results and Discussion

The chlophyll content in the two cultivars based on water regime was not significantly different but the values differ (Figure 1). The concentration of chlorophyll A was noticed to be higher than chlorophyll B in all the water regimes and cultivars with 600 m^3/mu HY8 having the highest value (6.19 mg/L) in 2012. The chlorophyll content in HY8 was lowest (5.54 mg/L) in the 300 m^3/mu in 2012. In 2013 however, the chlorophyll A content was highest (6.88 mg/L) in HY8 rolled leaf and followed by 300 m^3/mu HYXQ (6.54 mg/L). The same trend was observed in chlorophyll B content.

The proline content in the two cultivars and water regimes were noticed to be highest in the 300 m^3/mu in both years (Figure 1) with 2013 having the highest (6159.88; 6026.65 µg/g) values in the 300 m^3/mu rolled leaf. The proline content was higher in 600 m^3/mu (160.40 µg/g) than in 900 m^3/mu (118.60 µg/g) HY8 in 2012 in both cultivars while in 2013, 900 m^3/mu (237.20 µg/g) had a higher proline content than both 450 (228.84 µg/g) and 600 m^3/mu (222.57 µg/g).

The soluble sugar content in the cultivars and different water regime is present in Figure 1. The soluble sugar content was significantly higher 300 m^3/mu HY8 (0.472%) in 2012 than in the other water regimes. This was followed by HYXQ (0.338%) of the same water regime. The same trend was noticed in 2013 in both cultivars in the 300 m^3/mu (13.676; 10.143%). It was surprising to notice the low sugar content in the rolled leaves (4.704; 8.833%) in both cultivar which is almost similar to that in the higher water regimes.

It was noticed from the result that the protein content in the 300 m^3/mu (8.22; 8.04 mg/g) for both cultivars were higher compared to the higher water regime in 2012 (Figure 2). In 2013, the same trend was observed in the cultivars and water regimes with the rolled leaves from 300 m^3/mu (24.72; 22.43 mg/g) having the highest content followed by the normal leaf samples from the same water regime.

MDA concentration in the leaf sample from both cultivars in the 300 m^3/mu was significantly highest (Figure 2) in both years depicting the accumulation of the naturally occurring product of lipid peroxidation. High MDA concentration in plant system is known to be an indication of oxidative stress and an accumulation of ROS.

In 2012, the peroxide concentration in the plants was noticed to be higher at higher water regime but in 2013 sampling, the reverse was the case (Figure 2). The peroxide content was significantly higher in the rolled leaves and normal leaves of the 300 m^3/mu. This observation might be due to the difference in weather in the two years as plants are sessile in nature and always respond to stimuli around them.

Concentration of sodium dismutase (SOD) in the 2012 leaf samples showed a reverse trend as the catalase. The SOD concentration in the 300 m^3/mu was significantly lower (Figure 3) than in the other water regimes. This is feasible as SOD is a known scavenging substance of ROS and it is expected to be lower in the presence of accumulating ROS. In 2013 however, the SOD content was noticeably higher in the rolled leaves with the lowest content found in 600 m^3/mu HYXQ.

Both relative water content and water loss were observed to be low in 300 m^3/mu HYXQ and this was followed by HY8 in the same water regime. There were no significant differences between the relative water content and water loss in the 900 and 600 m^3/mu (Figure 3).

The catalase (CAT) content in the leaves from the 300 m^3/mu were observed to be relatively higher (Figure 3) than that in the other water regimes in 2012 while in 2013, no definite pattern was noticed. Different concentrations were noticed in the different cultivar under different water regime. The highest catalase value was found in the 600 m^3/mu HYXQ followed by both cultivars under 900 m^3/mu while the content in the rolled leaves were even lower.

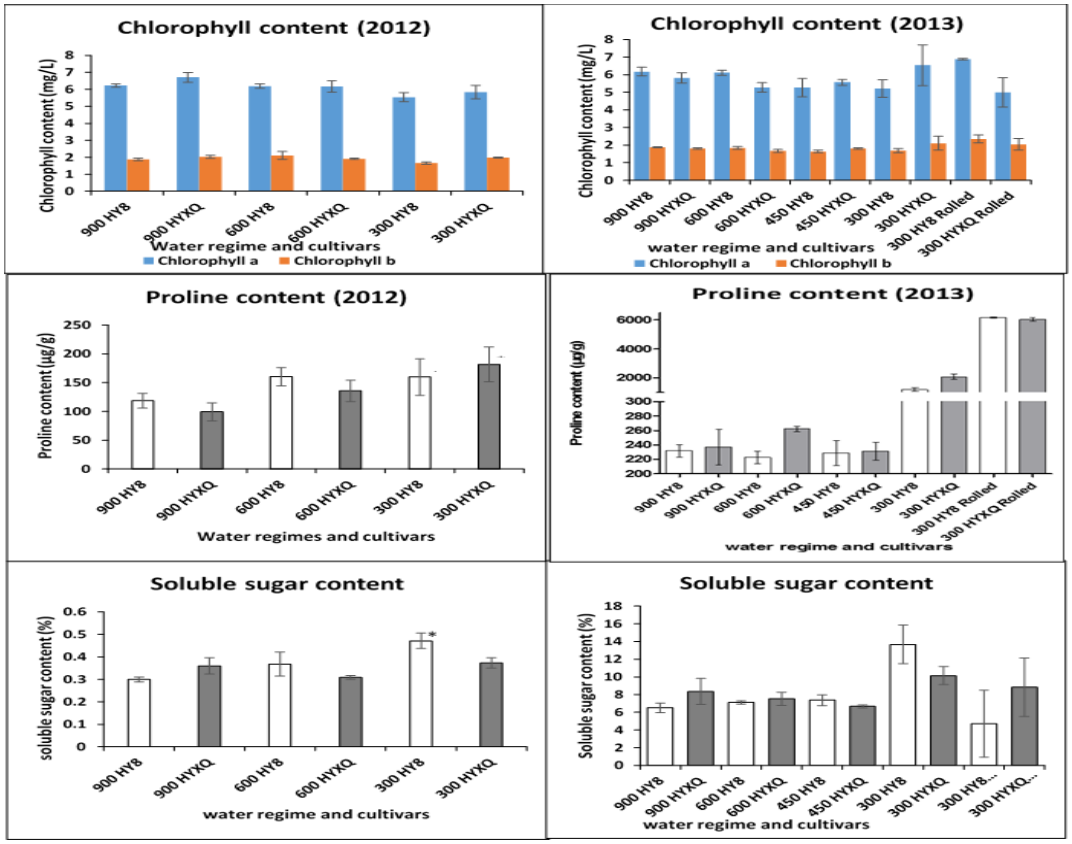

Figure 1: Effect of water regime on the chlorophyll, proline and soluble sugar contents the two cultivars

Figure presents the chlorophyll content, proline and soluble sugar contents in the two cultivars as affected by the water regimes in the two years. Error bars are based on 3 replications

Conclusion

ROS have been noticed to accumulate in plants under abiotic stress. The plant accumulation of these biochemical substances alters the normal function of the plant internal organs. Some of these substances when in high concentration become toxic to the plant system thereby causing a collapse of the plant system leading to spontaneous death. Plants are sessile in nature and respond to stimuli around them. In the event of stress, plants produce ROS which are highly reactive and toxic, and whose accumulation in the plant can cause oxidative cell destruction (Larkindale and Huang, 2004; Wormuth *et al*., 2007; Noctor and Foyer, 1998; Savicka and Škute, 2010, Blokhina, 2000). ROS substance in the plant cell include proline, peroxidase (POD), catalase (CAT), superoxide dismutase (SOD) and the product of lipid peroxidation – MDA.

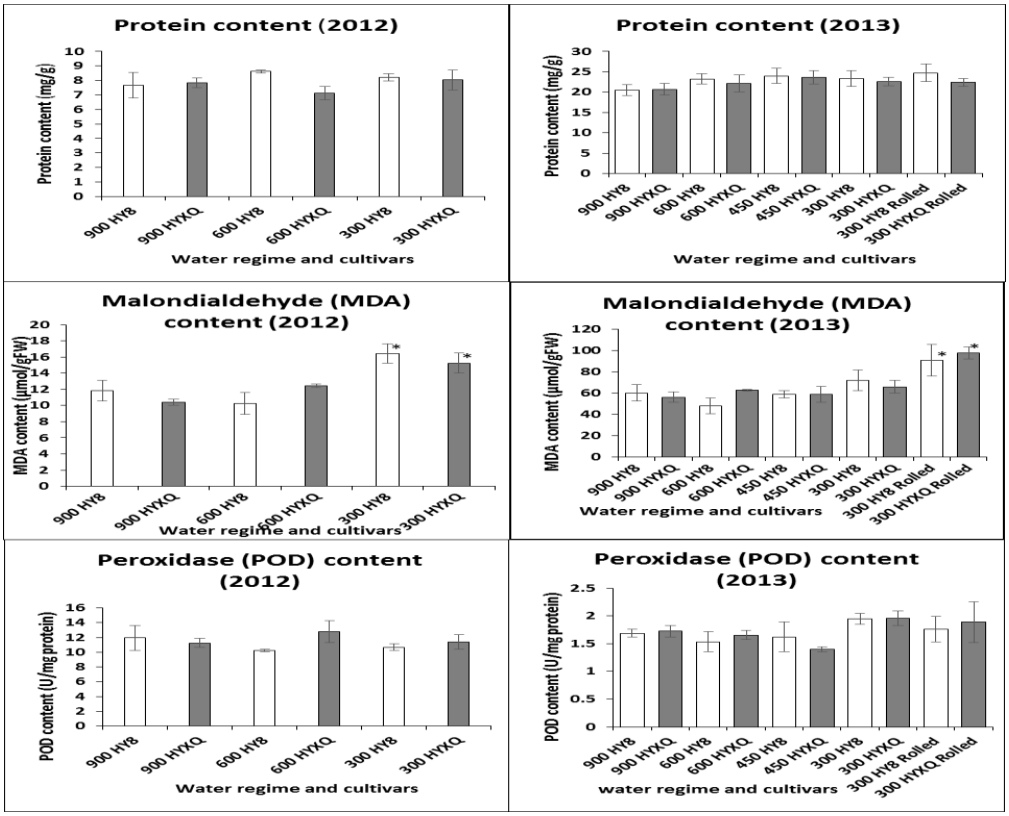

Figure 2: Effect of water regime on the protein, malondialdehyde and peroxide contents in the cultivars

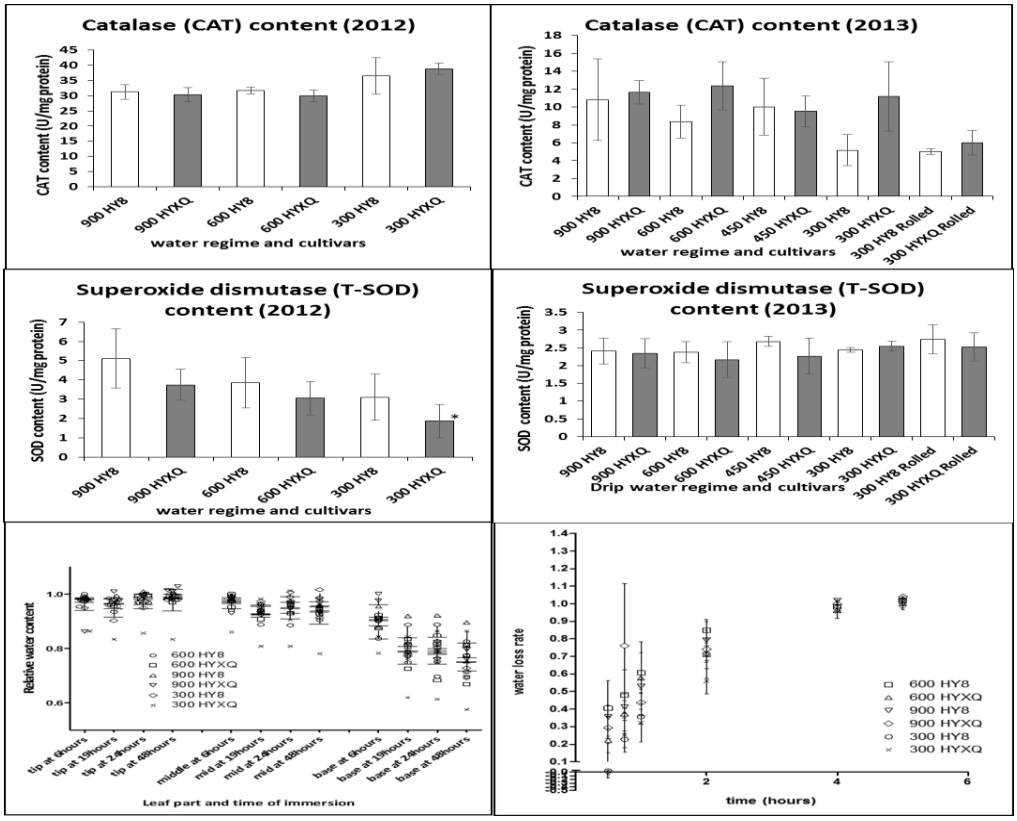

Figure 3: Effect of water regimes on the catalase, superoxide dismutase, relative water content and water loss in the two cultivars

Proline and MDA contents were noticed to be high in stressed plants in this experiment and consistent in the two years although the values were not exactly the same. This agreed with many reports on the effect of abiotic stresses on physiological activities of the

plant cell (Mittler, 2002; Demiral and Türkan, 2005; Amirjani, 2010; Savicka and Škute, 2010). Soluble sugar and protein content were observed to be higher in lower water regime while chlorophyll, POD and CAT content had no definite pattern among the treatments and cultivars. Demiral and Türkan, 2005 reported increase proline and MDA content in the root of two rice cultivars exposed to salt stress in addition to decrease in root fresh and dry weight. Salt stress is identical to drought stress as both lead to dehydration in which the plant water status is disturbed and imbalance leading to the activation of oxygen species (Demiral and Türkan, 2005). Amirjani, 2010 experienced the same trend while working on rice exposed to salinity stress. He observed increase in MDA and peroxide as well as reduced relative water content in the stressed plants. This corroborate with the finding from this study as the relative water content was lowest in the lowest water regime in both cultivars. Savicka and Škute, 2010 reported that increase in reactive oxygen (O^{-}_{2}) production which was observed in the first leaf of wheat seedlings at all stages of development, led to an increase of MDA concentration. Proline and MDA were noticed to be a spontaneous measure of effect of drought on plant physiological activities.

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