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Ameliorative Potentials of *BABA* on the Oxidative Stress Attributes of *Ofada* Rice Plant Subjected to Water Stress at the Vegetative Growth Stage

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ABSTRACT

Ofada rice (Oryza sativa L.) is an extremely sensitive rice variety to drought stress. The study was aimed at utilizing the potentials of BABA to ameliorate the oxidative stress in Ofada rice subjected to water deficit stress at the vegetative growth stage of the plant. Field experiments was carried out at the University of Ilorin teaching and research farm during the dry season. The experimental setup followed a complete randomized block design with five treatments and four replications. Ofada rice plants were subjected to four concentrations of beta-aminobutyric acid (BABA) treatments (0, 150, 300 and 600 µM) upon stress imposition at the vegetative stage of the plant growth, a full blown water regime was included as the positive control which is the fifth treatment. The result showed that BABA was able to delay the morphological manifestation of water stress in Ofada rice for a period of 12 days. Furthermore, a significant increase in superoxide dismutase, catalase, ascorbate peroxidase and nitrate reductase activity were also recorded in BABA treated water-stressed plant when compared to the negative control (0 µM BABA). All BABA treated water-stressed plants also showed a marked decrease in superoxide radical and hydrogen peroxide concentration as well as lipid peroxidation levels. Therefore, foliar application of BABA at the rate of 150- 300 µM should be incorporated in the cultivation of Ofada rice in the drought prone regions.

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1.0 Introduction

Water deficit stress is a peculiar constraint to the cultivation of rice crop globally most especially the arid and semiarid region[1]. In Nigeria, one of the major challenge faced by rice production farmers is insufficient water supply, especially during periods rainfall inadequacy coupled with frequent dry spells[2]. Rice leaves have a well-documented challenge of high transpiration rate [3] which increases the plants sensitivity to drought sequel to inability to regulate its water loss to transpiration as effectively as other cereals [4]. Drought-sensitive rice plant experiencing water deficit stress becomes damaged on an account of low tissue water potential [5] which consequently reduce the leaf surface area [6], photosynthesis, stomatal conductance, starch metabolism[7]. and an overall alteration in assimilate partitioning among the plant organs [8]. Biochemically, water deficit stress distorts the natural balance between cellular free radical production and their detoxification. Accumulation of undetoxified ROS result in oxidative stress which often leads to low crop yield and in severe cases, it leads to the death of the plant [9]. The Nigerian southern guinea savanna is characterized with a poor rainfall distribution which is less than 900 mm per annum, often interspersed with periods of dry spells [10]. This poor climatic condition is a factor limiting the cultivation of *Ofada* rice to the southern region of the country despite its high demand given its unique taste, natural flavour, higher fibre content and a higher nutritive value compared to polished rice[11]. Beta-aminobutyric acid (*BABA*) has a well-documented literature for its ability to induce resistance to abiotic stress in several plants by priming their antioxidant defense mechanism[12]. The present study therefore seeks to establish a possible novelty of utilizing *BABA* to enhance the stress tolerance level of *Ofada* rice plant subjected to water deficit stress at the vegetative growth stage of the plant perhaps it may survive the drought prone regions of the country.

2.0 Materials and Method

2.1 Experimental site description

A field experiment was conducted during the 2016 dry season at the University of Ilorin Teaching and Research Farm located at longitude 40 38.9201 E - 40 39.9711 E and latitude 80 27.8101 N - 80 28.2301 N in the Southern Guinea Savanna region of Nigeria.

2.2 Experimental Layout, Treatment Details and Agronomic Management

The study was designed as a complete randomized block comprising of five treatments and four replicates including positive control (full blown water regime), negative control (0 µM *BABA*), 150 µM *BABA*, 300 µM *BABA* and 600 µM *BABA*. The experimental plot measured 17.0 m × 13.5 m containing 20 net plots each measuring 3.0m x 3.0 m with 0.5 m alley between them. Two to four (2-4) viable seeds of *Ofada* rice (Straw red) was sown by directed seeding method where holes were drilled to about 3-4 cm depth with a spacing of 25cm by 25 cm. After the plant's establishment, it was thinned to one plant per stand. The plant was watered to 100% field capacity using 7,500 L of water at 48 hrs interval throughout the experiment. Hand weeding was done at intervals of two weeks from the onset of the experiment to the period of harvest. Water deficit stress was imposed on all treatment plots (except the positive control) by completely withholding irrigation water supply for a period of 14 days which commences at 6 weeks after planting (6 WAP). The foliar application of *BABA* was done 24 hours before water deficit stress imposition.

2.3 Determination of Plant Water Status

Relative water content (RWC) was determined by excising healthy intact leaves from each plot and the fresh weight (FW) was immediately recorded. Thereafter, the leaves were immersed in distilled water for twenty-four hours (24 hrs) to obtain full turgidity, excess surface water was removed by bloating and then the turgid weight (TW) was recorded. This was followed by a progressive drying of the leaf samples at 60°C for 24 hrs in an oven to obtain a constant dry weight (DW). The RWC was calculated using the formula:

$$RWC\ (\%) = \frac{(FW - DW)}{(TW - DW)} \times 100$$

Where Fw is fresh weight; DW: dry weight; TW: turgid weight.

Days to physical manifestation of water stress was determined by counting the number of days from stress imposition to the number of days in which 50 Percent of the plants per plot have manifested leaf rolling symptoms. Days to physical recovery from water stress was determined by counting the number of days from stress withdrawal to the number of days in which fifty Percent of the plants per plot have reversed the leaf rolling symptom which was manifested during water stress.

2.4 Preparation of Sample Homogenate for Biochemical Studies

The enzyme extract was prepared by homogenizing 500 mg of fresh leaf tissue in 2.5 mL of 0.05 M potassium phosphate buffer (pH 7.0). The homogenate was filtered and subsequently centrifuged at 15,000 × g for 30 min in a refrigerated centrifuge (Remi Instruments C-24). An aliquot (100 µL) of the resulting supernatant was transferred into an Eppendorf tube and diluted to a final volume of 1,000 µL with 50 mM potassium phosphate buffer (pH 7.0). The extract was stored at 4 °C and used for subsequent biochemical analyses.

2.5 Determination of free radical accumulation

Superoxide anion radical concentration in plant leaves was evaluated according to[13]. In a microplate of 300 µL well capacity, 100 µL of the sample homogenate was added to 50 µL of 1 mg/mL Nitroblue tetrazolium (NBT), the mixture was incubated at 370C for 30 mins thereafter, 10 µL of 0.1 M HCL was added and it was centrifuged at 1,500 g for 10 mins. DMSO was added to extract NBT and 60 µL of phosphate buffered saline(pH7.4) The same procedure was reproduced for calibration curve formulation with 100 µL H2O2, in a uniform order of decreasing concentration the sample homogenate was replaced by 100 µL distilled water. Absorbance was read on a microplate reader (UV max Kinetic) at 575 nm. Superoxide anion radical concentration was computed using the formula:-

$$\text{Superoxide radical concentration} = \frac{\text{Change in absorbance}}{\text{Extiction coefficient}} \times \text{Dilution factor}$$

Where extinction coefficient = 17,000 M-1 cm-1

Hydrogen peroxide was assayed for by adding 100 µL of the sample homogenate to 100 µL of 0.1% trichloroacetic acid (TCA). The resultant mixture was centrifuged for 20 minutes at 10,000 revolutions per minute, and the supernatant was extracted while the pellet was discarded. In a new 300 µL micropipette 50 µL of the supernatant collected from centrifugation was mixed with 50 µL of 10 mM potassium phosphate buffer, lastly 100 µL of 1M potassium iodide was added. The same procedure was repeated for the development of calibration curve but however, the sample homogenate was replaced by 100 µL each of a set of decreasing H2O2 concentration [14].

2.6 Determination of Antioxidant Enzymes Activities

Superoxide dismutase (SOD, EC 1.15.1.1) activity of the plant was assayed by monitoring the percentage inhibition of epinephrine oxidation according to[15]. In a 300 µL round-bottomed well microplate, 125 µL of 0.05M carbonate buffer (pH 10.2) was added to100 µL of the sample homogenate and 15 µL of 0.3 mM epinephrine using a multi-channel micropipette, the same procedure was repeated for the blank except for the fact that the sample homogenate was replaced by 100 µL of sucrose solution. The absorbance was read on a microplate reader (UV max Kinetic) at 480 nm at an interval of 30 s for 150 s. The percentage inhibition of epinephrine was calculated according to the formular:- $\% \text{ inhibition} = 100 - \left[\frac{\text{increase in absorbance of sample}}{\text{increase in absorbance of blank}}\right] \times 100$

Catalase (EC1.11.1.6) activity was evaluated in-vitro by determining the rate of disappearance of hydrogen peroxide (H2O2) per min at 374 nm [16], 50 µL of hydrogen peroxide prepared by mixing 500 µL of 20 mM H2O2 in 500 µL of 0.05 M sodium-potassium phosphate buffer at a pH of 7.4 was added to 5 µL of sample homogenate, the resulting mixture was incubated at 37°C for 3 mins, thereafter 200 µL of 32.4 mM ammonium molybdate was added for colour development. The same procedure was repeated for the standard and control test samples however, the sample homogenate was replaced by 5 µL distilled water in the former while 50 µL H2O2 was missing in the later. The catalase activity was deduced according to the formular:-

$$\text{Catalase activity} = \frac{2.303}{t} \times \left[\log \frac{S^0}{S - M}\right] \times \frac{Vt}{Vs}$$

where S° = Absorbance of the standard tube; t = time; S = absorbance of test tube; M = Absorbance of control test (correction factor); Vt = Total volume of reagents in the microplate; and Vs = Volume of sample homogenate.

Ascorbate peroxidase (EC1.11.1.11) activity was determined measuring the amount of enzyme required to oxidise 1µM ascorbate min-1 as described in the method of[14] with slide modification. to). To a 300 µL round-bottomed microplate, 225 µL of 50 mM potassium phosphate buffer (pH 7.4) was mixed with 0.5 µL of 0.1 M ascorbate, thereafter, 7.5 µL of sample homogenate and 5 µL of 10 mM H2O2 were added and the absorbance of the resulting mixture was read on a microplate reader (UV max Kinetic) at 340 nm at an interval of 30 s for 180 s. The enzyme activity was then calculated using the formula:-

$$\text{Enzyme activity} = \frac{\text{Change in absorbance} \times 2Vq}{2.8 \times Vs}$$

where Vq = total volume of the reaction mixture; Vs=Volume of sample

2.7 Determination of Oxidative Stress Attributes

Lipid peroxidation was measured by estimating the malondealdehyde content (MDA) following[17], 50 µL of the sample homogenate was added to 100 µL of 0.37%/15%/0.2N TBA/TCA/HCL prepared by mixing the three reagent in 1:1:1, for the blank, the sample homogenate was replaced by 50 µL standard sucrose solution. The resultant mixture was boiled on water bath for 15 minutes, allowed to cool at room temperature, and centrifuged thereafter for 10 minutes at 1000 revolution per minute. The optical density of the solution was recorded at 535 nm against the blank. Thereafter, the MDA content was calculated according to the formular:-

$$\text{MDA content} = \frac{\text{Change in absorbance}}{\text{Exticion coefficient}} \times \text{ Dilution factor}$$

where the extinction coefficient = 156,000 M-1cm-1

Chlorophyll content was evaluated within the period of the plant's exposure to water deficit stress. Chlorophyll was extracted on the flag leaf of plants according to the methods of[18]. One gram of the fresh leaf tissue was extracted in 10 mL of absolute ethanol in placed in a non-transparent specimen bottle for a period of two weeks. Thereafter, 1 mL of the extract was transferred in to a new test tube and it was made up to 7mL using absolute ethanol and then transferred in to the cuvette of a spectrophotometer where the absorbance was read against ethanol blank at 645 and 663nm respectively. Chlorophyll a and b content in milligrams of chlorophyll per gram of leaf tissue were determined according to[19] using the following formula:-

Chlorophyll A (mg/g leaf tissue) = 12.7(D663)-2.67(D645) x V/1000 x W

Chlorophyll B (mg/g leaf tissue) = 22.9(D645)-4.68(D643) x V/1000 x W

Where D = Absorbance at wavelengths 645 nm and 663 nm, V = Volume (mL) of the ethanol extract, W = Fresh weight of leaf tissue

Nitrate reductase activity (NRA) was determined as a function of nitrite formed at the end of the reaction. Exactly 20 µL of sample homogenate was added to 20 µL of 20 mM sodium nitrate in a microplate (Dynatech Labs Inc., Chantilly, VA). Thereafter, the resulting mixture was incubated at 37°C for 20 minutes, this was followed by the termination of the reaction by addition of 20 µL of 1% sulfanilamide (prepared in 10% TCA) and cooled on ice for 2 mins. The reaction mixture is centrifuge for 1 minutes at 6,000 g where precipitate was formed. Thereafter, 20 µL of the supernatant was transferred to a clean microplate and 20 µL of 0.1% N (1-naphthyl) ethylenediamine was added for colour development. Following the incubation of the resultant mixture for 30 minutes at room temperature, the optical density (OD) of all samples were measured at 560 nm using a microplate reader (UVmax Kinetic). Free proline content was determined following the method of [20]. Leaf samples (250 mg) were homogenized in 3% (w/v) sulphosalicylic acid and centrifuged at 4,000 rpm for 15 min. The resulting supernatant was reacted with acid ninhydrin reagent, prepared by dissolving 1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL of 6 M orthophosphoric acid. The reaction mixture was heated in a boiling water bath for 1 h, followed by extraction with 4 mL toluene. The absorbance of the toluene-rich chromophore was measured at 520 nm. Free proline content was expressed as µmol g^{-1} fresh weight (FW).

3.0 Results

3.1 Effect Of *BABA* on The Plant Water Status of Water Stressed *Ofada* Rice Plant

Generally, the relative water content (RWC) was significantly the same at 0 days post drought induction (DPDI) (Table 1). Thereafter, the negative control plant recorded significant ($p<0.05$) lowest RWC at 7 and 14 DPDI while *BABA* treated drought stressed plants had higher RWC compared to the negative control. Also *BABA* treated plants delayed the morphological manifestation of drought by12 days while same was manifested in the negative control plants within 5 days of water stress imposition (Table 2). The negative control plants also recorded significant ($p<0.05$) highest number of days to recovery (4 days) as opposed to 2 days in *BABA* treated drought stressed plants (Table 2).

3.2 Effect of *BABA* on Reactive Oxygen Species Concentration of Water Stressed *Ofada* Rice Plant

Significant ($p<0.05$) highest superoxide anion radical and hydrogen peroxide was recorded in the negative control plants at 7 and 14 DPDI while *BABA* treated drought stressed plants had a significant ($p<0.05$) lower reactive oxygen species (ROS) accumulation when compared to the negative control (Table 3). However, the positive control (full blown water regime without *BABA*) recorded a significant ($p<0.05$) lowest ROS accumulation compared to all other treatments considered in the study (Table 3).

3.3 Effect of *BABA* on The Antioxidant Enzyme Activities of Water Stressed *Ofada* Rice Plant

Generally, superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) were significantly ($p<0.05$) the same for all treatments considered at 0 DPDI (Table 4). Thereafter, the aforementioned antioxidant enzymes levels were elevated significantly in *BABA* treated drought stressed plants as opposed to a significantly ($p<0.05$) lower antioxidant enzyme activity recorded in the negative control when compared to the *BABA* treated plants (Table 4)

3.4 Effect of *BABA* on The Oxidative Stress Attributes of Water Stressed *Ofada* Rice Plant

At 0 DPDI, malondialdehyde (MDA), total chlorophyll content, nitrate reductase activity (NRA), and proline content did not differ significantly ($p < 0.05$) among the treatments evaluated (Table 5). Subsequently, the negative control plants exhibited a significantly higher MDA content compared with all other treatments. In contrast, drought-stressed plants treated with BABA showed a significantly lower MDA content relative to the negative control (Table 5). Moreover, the negative control plants recorded significantly lower ($p < 0.05$) total chlorophyll content, NRA, and proline levels compared with all other treatments. Conversely, BABA-treated drought-stressed plants exhibited significantly higher total chlorophyll, NRA, and proline contents than the negative control.

Table 1 :- Effect of *BABA* on the relative water content (%) of drought stressed *Oryza sativa*

Treatments	0 Days PDI	7 Days PDI	14 Days PDI	7 Days PDW
T_0	91.46±1.40	92.13±1.12 ^{a}	94.16±0.17 ^{a}	91.04±1.88
T_1	93.35±1.26	62.26±0.74 ^{c}	48.23±1.33 ^{d}	87.69±1.64
T_2	93.83±1.77	86.17±1.24 ^{b}	73.21±1.74 ^{b}	89.08±1.68
T_3	93.13±1.62	83.90±0.68 ^{b}	70.80±1.95 ^{b}	88.51±2.15
T_4	93.20±1.23	82.86±1.45 ^{b}	65.95±1.64 ^{c}	86.61±2.00
Total	93.00±0.60	81.46±2.74	70.47±3.98	88.59±0.81
p-value	0.82	<0.01	<,0.01	0.56

Values are mean± SEM, n=4, values with same superscript across the treatments are not significant at p<0.05; T_0=positive control, T_1=negative control, T_2=150µM, T_3=300µM, T_4=600µM; PDI: post drought induction; PDW: post drought withdrawal, *BABA*:- beta aminobutyric acid.

Table 2:- Effect of *BABA* on drought manifestation and recovery of *Oryza sativa*

Treatments	DTMMD	DTPR
T_0	------	------
T_1	5.67±0.33 ^{c}	4.33±0.33 ^{a}
T_2	12.33±0.33 ^{a}	2.00±0.00 ^{b}
T_3	12.00±0.00 ^{a}	1.67±0.33 ^{b}
T_4	10.33±0.33 ^{b}	1.67±0.33 ^{b}
Total	8.07±1.26	1.93±0.38
p-value	< 0.01	< 0.01

Values are mean± SEM, n=4, values with same superscript across the treatments are not significant at p<0.05; T_0=positive control, T_1=negative control, T_2=150µM, T_3=300µM, T_4=600µM; DTMMD: days to morphological manifestation of drought; DTPR:- days to plant recovery; *BABA*:- beta aminobutyric acid.

Table 3:- Effect of *BABA* on free radical accumulation of drought stressed *Ofada* rice leaves

Free radicals (µmol.mg^{-1} Fw)	Treatments	0 Days PDI	7 Days PDI	14 Days PDI	7 Days PDW
Superoxide anion	T_0	0.30 ±0.03	0.31±0.01 ^{c}	0.30±0.03 ^{c}	0.33±0.02 ^{b}
	T_1	0.31 ±0.05	0.96±0.01 ^{a}	1.87±0.06 ^{a}	0.79±0.05 ^{a}
	T_2	0.30 ±0.03	0.52±0.07 ^{b}	0.41±0.01 ^{b}	0.35±0.02 ^{b}
	T_3	0.30 ±0.03	0.51±0.05 ^{b}	0.42±0.02 ^{b}	0.34±0.01 ^{b}
	T_4	0.30 ±0.03	0.54±0.09 ^{b}	0.47±0.03 ^{b}	0.33±0.02 ^{b}
	Total	0.30±0.01	0.57±0.06	0.70±0.16	0.43±0.05
	p- value	0.99	< 0.01	< 0.01	< 0.01
Hydrogen peroxide	T_0	0.17±0.01	0.15±0.01 ^{c}	0.11±0.00 ^{d}	0.15±0.02
	T_1	0.17±0.01	0.42±0.04 ^{a}	0.68±0.02 ^{a}	0.13±0.00
	T_2	0.16±0.01	0.25±0.02 ^{bc}	0.25±0.01 ^{c}	0.12±0.01
	T_3	0.15±0.01	0.26±0.02 ^{b}	0.26±0.01 ^{c}	0.12±0.01
	T_4	0.17±0.01	0.30±0.02 ^{b}	0.34±0.01 ^{b}	0.14±0.01
	Total	0.17±0.00	0.28±0.02	0.33±0.05	0.13±0.01
	p-value	0.81	< 0.01	< 0.01	0.05

Values are mean± SEM, n=4, values with same superscript across the treatments are not significant at p<0.05; T_0=positive control, T_1=negative control, T_2=150µM, T_3=300µM, T_4=600µM; PDI: post drought induction; PDW: post drought withdrawal, *BABA*:- beta aminobutyric acid

Table 4:- Effect of *BABA* on the antioxidant enzyme activity of drought stressed *Ofada* rice leaves

Eenzyme activity (µmol. Min^{-1}.mg^{-1} protein)	Treatments	0 Days PDI	7 Days PDI	14 Days PDI	7 Days PDW
Superoxide dismutase	T_0	16.21±1.19	15.91±1.84^{d}	15.83±2.32^{b}	15.44±1.69
	T_1	15.65±1.72	22.35±0.55^{c}	19.23±0.85^{b}	15.44±1.07
	T_2	16.74±1.52	32.52±2.03^{a}	32.15±2.19^{a}	15.36±1.44
	T_3	16.48±1.95	31.64±2.94^{a}	32.84±2.00^{a}	15.48±1.24
	T_4	16.19±1.57	25.50±0.67^{b}	29.36±1.13^{a}	15.51±1.58
	Total	16.33±0.67	25.58±1.78	25.88±1.99	15.45±0.54
	p- value	0.98	< 0.01	< 0.01	1.00
Catalase	T_0	2.08±0.01	1.96±0.03^{d}	2.00±0.01^{b}	2.04±0.03
	T_1	2.02±0.03	2.89±0.11^{c}	1.65±0.07^{b}	1.96±0.06
	T_2	2.05±0.01	3.98±0.28^{a}	6.22±0.24^{a}	2.05±0.01
	T_3	2.05±0.02	3.74±0.19^{ab}	6.32±0.17^{a}	2.01±0.00
	T_4	2.05±0.02	3.32±0.11^{bc}	6.31±0.23^{a}	1.99±0.04
	Total	2.05±0.01	3.18±0.20	4.50±0.59	2.01±0.02
	p-value	0.29	<0.01	<0.01	0.33
Ascorbate peroxidase	T_0	17.29±0.53	18.00±0.34^{c}	17.73±1.42^{b}	18.52±1.49
	T_1	17.79±0.02	20.97±0.96^{bc}	19.84±0.79^{ab}	19.46±0.47
	T_2	17.61±0.53	25.43±1.70^{a}	22.90±0.86^{a}	19.31±1.17
	T_3	17.41±0.50	24.12±1.39^{ab}	21.92±0.89^{a}	18.72±1.02
	T_4	17.80±0.19	27.60±0.40^{a}	22.49±0.67^{a}	19.68±0.84
	Total	17.58±0.17	23.23±0.99	20.98±0.63	19.14±0.42
	p-value	0.87	<0.01	0.02	0.92

Values are mean± SEM, n=4, values with same superscript across the treatments are not significant at p<0.05; T_0=positive control, T_1=negative control, T_2=150µM, T_3=300µM, T_4=600µM; PDI: post drought induction; PDW: post drought withdrawal, *BABA*:- beta aminobutyric acid

Table 5:- Effect of *BABA* on oxidative stress attributes of drought stressed *Ofada* rice leaves

Oxidative stress attributes	Treatments	0 Days PDI	7 Days PDI	14 Days PDI	7 Days PDW
Malondealdehyde content (µmol.mg^{-1} Fw)	T_0	2.70±0.04^{a}	2.60±0.07^{c}	2.78±0.12^{c}	2.97±0.09^{c}
	T_1	2.78±0.04^{a}	6.45±0.06^{a}	8.18±0.14^{a}	7.89±0.09^{a}
	T_2	2.72±0.04^{a}	3.35±0.04^{b}	3.50±0.08^{b}	3.45±0.08^{b}
	T_3	2.69±0.06^{a}	3.40±0.08^{b}	3.55±0.09^{b}	3.50±0.04^{b}
	T_4	2.63±0.10^{a}	3.41±0.09^{b}	3.61±0.08^{b}	3.51±0.07^{b}
	Total	2.70±0.03	3.85±0.36	4.32±0.52	4.26±0.49
	p- value	0.59	<0.01	<0.01	<0.01
Total Chlorophyll content (mg.g^{-1} Fw)	T_0	141.49±3.03	149.11±2.14^{a}	144.37±2.70^{a}	146.11±3.40^{a}
	T_1	141.60±3.13	128.24±1.31^{c}	109.61±2.71^{c}	114.92±8.80^{b}
	T_2	138.62±4.70	141.12±3.49^{b}	130.82±2.80^{b}	145.39±2.54^{a}
	T_3	138.00±2.11	140.97±3.60^{b}	129.24±1.80^{b}	142.03±3.81^{a}
	T_4	142.98±2.39	138.70±2.06^{b}	127.77±3.84^{b}	142.38±5.49^{a}
	Total	140.54±1.31	139.63±2.06	127.56±2.70	138.17±3.25
	p-value	0.77	0.04	< 0.01	< 0.01
Nitrate reductase Activity (µmol. Min^{-1}.mg^{-1} protein)	T_0	35.07±0.66	34.30±1.68^{a}	34.19±2.03^{a}	29.67±1.01^{a}
	T_1	32.31±0.77	19.95±1.36^{b}	15.07±0.65^{c}	17.13±0.89^{b}
	T_2	34.62±1.15	29.59±1.59^{a}	25.17±1.04^{b}	28.22±1.58^{a}
	T_3	34.24±1.54	29.55±1.71^{a}	25.63±1.86^{b}	27.70±1.85^{a}
	T_4	34.02±1.61	30.72±2.25^{a}	25.35±1.28^{b}	27.93±0.26^{a}
	Total	34.05±0.52	28.83±1.43^{a}	25.081.71	26.131.30
	p-value	0.58	0.02	<0.01	<0.01
Proline accumulation (µmol.mg^{-1} Fw)					
	T_0	27.17±1.14	26.82±1.46^{b}	26.78±2.40^{c}	26.82±0.35
	T_1	27.84±1.21	33.09±1.45^{b}	39.02±0.88^{b}	28.79±0.88
	T_2	26.85±0.68	51.25±2.10^{a}	70.92±1.76^{a}	28.12±1.52
	T_3	27.82±1.84	49.59±2.35^{a}	69.60±3.92^{a}	26.79±1.76
	T_4	26.18±1.52	48.97±3.76^{a}	72.01±2.39^{a}	28.48±0.66
	Total	27.17±0.53	41.94±2.82	55.66±5.16	27.80±0.49
	p-value	0.89	<0.01	<0.01	0.60

Values are mean± SEM, n=4, values with same superscript across the treatments are not significant at p<0.05; T_0=positive control, T_1=negative control, T_2=150µM, T_3=300µM, T_4=600µM; PDI: post drought induction; PDW: post drought withdrawal, *BABA*:- beta aminobutyric acid

4.0 Discussion

4.1 Effect of *BABA* on The Plant Water Status of Water Stressed *Ofada* Rice Plant

Significant decrease in the RWC of water stressed plant could be due to the water deficit created in the plant tissue as a result of the stress imposition. Significant lowest RWC recorded in the negative control is indicative of the plants sensitivity to water stress in the absence of any external ameliorative agent. Rice crop have been reported to exhibit the high sensitivity to water deficit resulting in a significant decrease in its RWC [21]. Significant decrease in the number of days to physical recovery from water stress in *BABA* treated plants when compared to the negative control could be adduced to *BABA* induced osmotic adjustment ability, maintenance of cell turgor which ultimately results in protection of cell membrane and other cellular organelles from damage [22]. This facilitate the recovery time upon withdrawal of water stress since the damage caused by the water deficit is minimal under the influence of *BABA*.

4.2 Effect of *BABA* on Free Radical Accumulation of Water Stressed *Ofada* Rice Plant

Superoxide radical and hydrogen peroxide are inevitable free radicals produced aerobic respiration in living cells[23]. Superoxide radical is generated as a result of incomplete reduction of molecular oxygen by leakages of electron to Oxygen in the electron transport chain[24] while hydrogen peroxide is generated from superoxide radicals on account of spontaneous dismutation [25]. However, both are toxic to the plant cells and must be scavenged rapidly by the antioxidative defence system to avoid oxidative stress[26]. Significantly higher concentration of the aforementioned free radicals in plant leaves subjected to drought at the vegetative stage is suggestive of the crop's sensitivity to water deficit stress. Plant sensitivity to drought have been positively correlated with free radical accumulation in plant cells[27]. Significant decrease in superoxide radical and hydrogen peroxide concentration of water stressed plant under various *BABA* treatment studied is indicative of *BABA* induced facilitation of free radical detoxification potentials of the plant[28]. This is evident in the higher SOD, CAT and APX, activity obtained in the leaf tissues of the *BABA* treated drought stressed plants. [29]have also reported a decrease in ROS concentration of *BABA* treated plants when compared to those without *BABA*. The result also aligns with the finding of[30] on drought tolerance level of three wheat cultivars.

4.3 Effect Of *BABA* on The Antioxidant Enzyme Activities of Water Stressed *Ofada* Rice Plant

Superoxide dismutase (SOD) is an essential component of the plants antioxidant defence system known for its ability to dismutate superoxide radicals to water and oxygen[31] A brief increase in the activity of SOD in rice plants growing under water stress could be adduced to a progressive soil drying during the early stage of the water deficit stress imposition. Increase in SOD activity has been implicated with a progressive water stress[32], salinity[33] gamma radiation[34], ultraviolent radiation[35] as well as heavy metal toxicity[36]. During the progressive stress, a stage is reached when the SOD enzymes becomes saturated and activity becomes constant giving room for accumulation of undetoxified free radicals in the plant tissue as evident in the higher concentration of free radicals in the negative control treatments. Significant increase in SOD activity of all *BABA* treated water-stressed plants at 7 and 14 days after stress imposition could be adduced to *BABA* mediated enhancement of the antioxidant activities. *BABA* have been used to effectively prime the antioxidant defense systems which ultimately increased wheat tolerance to desiccation[28]. Catalase (CAT) is an oxidoreductase which breaks down hydrogen peroxide to molecular oxygen and water[37]. Ascorbate peroxidase (APX) is an indispensable components of the ascorbate/glutathione pathway, required to eliminate potentially harmful hydrogen peroxide produced mainly in chloroplasts and other cell organelles by utilizing the reducing power of ascorbic acid[38]. Significant increase in CAT and APX activity in *BABA* treated water-stressed plant at 7 and 14 days after water stress imposition is indicative of *BABA* induced antioxidant enhancement which ultimately prevents the free radicals from accumulation[27]. *BABA* have been used to effectively prime the antioxidant defence systems which reduced water use and increased the desiccation tolerance in wheat[28]. Increased CAT and APX activity is evident in significantly lower hydrogen peroxide concentration of *BABA* treated plant as obtained in the present study.

4.4 Effect of *BABA* on The Oxidative Stress Attributes of Water Stressed *Ofada* Rice Plant

Lipid peroxidation is a measure of the malondealdehyde (MDA) content and it has been described as a function of ROS production and accumulation in plant tissue while reductions in chlorophyll content have been implicated as a consequence of oxidative stress resulting from lipid peroxidation. Free proline accumulation is an indication of reduced osmotic adjustment ability in plant due to water stress and its accumulates in an attempt to preserves the quaternary structure of complex proteins, maintains membrane integrity and stabilize sub-cellular structures[29]. Significantly higher level of lipid peroxidation in the negative control as the drought progress could be due to oxidative stress which resulted from the accumulation of undetoxified free radicals as the antioxidant enzymes becomes saturated and its activity becomes constant with increasing ROS production [27]. Significantly lower MDA content in *BABA* treated water stressed plant is indicative of a lower level of oxidative stress which could be adduced to enhanced ROS scavenging ability of the *BABA* treated plant as evident in their higher superoxide dismutase, catalase and ascorbate peroxidase activity when compared to the negative control[26]. Significantly lower chlorophyll content recorded in the negative control plants is suggestive of damages to the thylakoid membrane on account of oxidative stress [40]. Reductions in pigment content under stressful conditions have been identified as a consequence of oxidative stress on account of lipid peroxidation[41]. A significant decline in NR activity in water stressed plant could be attributed to low nitrate absorption and availability resulting from water uptake deprivation[42]. It could also be linked to a decrease in photosynthetic carbon assimilation on an account of stomatal closure since the energy and C skeletons required for N assimilation are provided by photosynthesis. High rate of CO_2 assimilation which favours a high rate of N assimilation has been positively correlated to NRA[43]. Significantly lower accumulation of proline in untreated water-stressed '*Ofada*' rice plant (negative control) is suggestive of the plants low tolerance to drought by default. Stress tolerant plants have higher proline content when compared to stress-sensitive plants[44]. Significantly higher proline concentration in *BABA* treated water-stressed plant when compared to the negative control could be indicative of effective osmotic adjustment and protective response as evident in lower level of lipid peroxidation and enhanced ROS detoxification system all of which contribute to the plants enhanced water deficit stress tolerance when compared to the negative control[26].

5.0 Conclusion

Generally, the effectiveness of foliar *BABA* application at 150-300µM as evaluated in this study was found to be significantly the same as all the aforementioned treatment rates were able to enhance the water stress tolerance level of *Ofada* rice plant through an induced delay in the physical manifestation of water stress effect and enhancement of the antioxidant defense system which lead to reduced oxidative damage as well as an improved physiological resilience. Therefore, foliar application of *BABA* at 150-300µM should be integrated into the cultivation of '*Ofada*' rice in the drought prone regions to ameliorate water deficit stress effects at the vegetative growth stage of the crop.

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