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Research Article

Physio-Chemical Properties Responses of Six Fonio Genotypes (*Digitaria exilis and D. iburua*) Subjected to Drought Stress Conditions

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Abstract
The cultivation of fonio in the Western Africa has been greatly hindered by the low amount of rainfall. Crop failure due to drought has been widely reported in Nigeria, which has led to research on different crops with paucity of information on fonio. The present study evaluated six drought genotypes of fonio to determine their physiological and biochemical responses under drought stress conditions. Fonio seeds were planted for a period of 3 weeks and subjected to drought. WUE decreased under drought stress particularly in the P21 genotype, while the RSR significantly increased for all evaluated genotypes. Drought stress seems to enhance the lipid peroxidation and free amino acid activities in the 6 evaluated genotypes, whereas it reduced the proline activities in 4 out of the 6 genotypes. At the early and the late stage, the highest lipid peroxidation was recorded in P25 and P21 respectively. The SOD activities varied at the early and the late stage under drought stress conditions, particularly for the P25, which showed higher level of SOD activities (= 10 folds higher than the control group at the late stage). The activities of CAT and GR for all genotypes studied were not affected by these stressful conditions. However, the APX activity was preferentially reduced in some genotypes, while the remaining ones were unchanged. In conclusion, difference in the physiochemical responses to drought reflects the differences in the ability of the genotypes to withstand varying degrees of drought. The increase in SOD activities might be a frontline defence to counter the increase observed in lipid peroxidation content caused by the reduction in CAT activities under droughtconditionsn.

Keywords: Fonio, Drought, Super-oxide dismutase, Catalase, Physiological, Biochemical.

Introduction

Drought is Africa's principal type of natural disaster. It is considered the most complex but least understood of all natural hazards affecting more people than any other one [1]. Droughts are frequent and severe in many African countries because of the extreme rainfall variability in the extensive arid and semi-arid areas and the poor capacity of most African soils to retain moisture. Over the past two decades, many sub-Saharan African economies are widely perceived as being particularly vulnerable to the effects of drought because of the importance of rain-fed agricultural production in GDP. According to SADCC report [2], the 1991–92 drought in southern Africa affected 20 million people and resulted in a deficit of cereal supplies of more than 6.7 million tons, while in West Africa, Crop failure due to drought was reported in the 1970s and 1980s [3]. In 1983 and 1984, the entire Sahel was impacted by a rainfall deficit that affected 80% of the yearly rainfall average in Nigeria [4], resulting in low food production. Nevertheless, insufficient cereal production was not always due to insufficient

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annual rainfall, but it was often due to intra-seasonal climate changes, as it was the case in Northern Nigeria at the outset of the 1979-1983 seasons [4].

The fonio crop has the capacity to contribute significantly to the overall whole grain feeding systems, the well-being, the economic development, and the security of the food supplies, particularly in the context of the drought situation that threatens the food security in the Sub-Saharan Africa Region (SSA). This variety of cereal has not received enough attention in comparison to the other major food cultures, especially because of a lack of data on its diversity [5]. Nowadays, the development of modern and appropriate fonio varieties, which can meet the needs of the farmers and the consumers regarding drought tolerance, seed size, and lodging tolerance, etc. are pressing requirements to deal with the continuously changing climate [6]. To respond to this threatening global food crisis and its serious consequences, it is necessary for countries of the sub-Saharan Africa to look inwards at indigenous resistant food crops, especially those neglected and underutilized ones, like fonio, due to influx of western foods into the continent. However, the yields of fonio have drastically reduced over many years across some growing locations in Nigeria because of drought problems [5].

Plant physiology has provided germane insights and developed new tools to understand the complex network of drought-related traits, while series of fundamental research has provided clearer understanding of the physiological responses of plants to drought stress. The physiologically relevant indicators of drought effects are the water content and water potential of plant tissues. Plant's response to drought stress has been studied in Arabidopsis, some dicots and certain grass species [7]. Unlike crops of worldwide importance such as maize, sorghum, cowpea, groundnut, and millet etc. which have several drought-resistant varieties, no effort has been made so far for a better understanding of the physiochemical mechanism of dehydration response that will contribute to the progress of genetic enhancement of drought resistant varieties in fonio. Plants are equipped with enzymatic and non-enzymatic natural defense systems against stress conditions (e.g., drought) that allow them to sequester and detoxify Reactive Oxygen Species (ROS) which can cause a variety of oxidative damage to the plant cell. When plants are exposed to various degrees of abiotic stresses, reactive oxygen species (ROS) such as superoxide radical (O), hydrogen Peroxide (H2O2), hydroxyl radical (OH) and singlet oxygen (^{1}O2) are produced. Mechanism of detoxifying ROS exist in plants, and it include activation of different enzymes (superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase). The degree to which responses of antioxidant enzymes are being triggered under drought stress is extremely variable among several plant species and even between cultivars of the same species [8].

The ability of plants to capture ROS and reduce their adverse effects may be related to their resistance to drought. In response to drought stress, plant antioxidant enzymes can either increase, decrease, or remain unchanged depending on the crop variety, duration of drought, and type of antioxidant [9]

This phenomenon of response to stress conditions, which several plant varieties such as fonio can be employed to counteract with, via antioxidant enzyme pathways, is not well investigated when plants are subjected to the same degree of drain and water deficiency. In fact, to date, there is limited information about the physiological response and the accumulation of antioxidant enzymes and their roles in drought adaptation of fonio.

The aim of the present study was to determine the different physiological and biochemical responses of six fonio genotypes, when they are exposed to drought stress conditions, and to compare the level of difference in their responses among the six genotypes studied.

Material and Methods

Source of plant materials

Seventy-five Germplasm were collected from Niger, Kaduna, Bauchi, Nasarawa and Plateau states in Nigeria. The germplasm was screened for drought tolerance in our previous study [5] and the following tolerant genotypes were identified: P19, P21, P22, P23, P25 and P29 (Table 1).

Experimental design

The experiment design was laid down in a Complete Randomized Design with five replications in the Department of Botany screen house, University of Lagos, Nigeria. Six genotypes of fonio were assessed for their Physio-chemical properties under drought stress.

Twenty seeds of each genotype were planted inside a container containing 800 g of loamy soil, equidistance (30 cm) from each other. The growing temperature ranged from 34.1 ° C during the daytime to 27.9 ° C at night, while maximum and minimum humidity were 73 % and 52 %,

respectively. Each of the fonio genotypes were watered regularly with equal volume (200 ml) of water for 21 days before the inducement of water stress and thinned to one plant after 21 days from planting date. The study was done in triplicates where progressive water deficit was applied. After 21 days of growth, drought was imposed for 4 and 7 days on each fonio genotypes. Physiological and biochemical properties were evaluated at day 4 (early) and day 7 (late) following their exposure to drought stress. For the control groups, the plants were well watered. Our previous reports [5] have reported day 7 of drought stress as the sensitive response day for drought stress in fonio. The container was kept free of weeds throughout the growing season.

Table 1.

List of drought tolerant genotypes used in this study

S/N	Identification Code	State	Local Govt	Species Name	Local Name	Coordinates		Altitude
1.	P19	Plateau	Pankshin	*D. iburua*	Shalak	N 9.38392	E 9.38120	1050 M
2.	P21	Plateau	Kanke	*D. iburua*	Gotip	N 9.25778	E 9.55120	850 M
3.	P22	Plateau	Kanke	*D. iburua*	Nashelleng	N 9.25778	E 9.55120	850 M
4.	P23	Plateau	Kanke	*D. iburua*	Jipel	N 9.25778	E 9.55120	850 M
5.	P25	Plateau	Bokos	*D. exilis*	Nyemat	N 9.18192	E 8.95595	1114 M
6.	P29	Plateau	Bokos	*D. iburua*	Black Jarab	N 9.12530	E 8.87650	1061 M

Evaluation of physiological properties

The relative water content of the excised leaf from tolerant genotypes was determined following [10]. The excised leaf was weighed adequately to determine the fresh weight (FW) and later immersed in double distilled water in the absence of light. After 24 h, it was weighed to determine the turgid weight (TW) and dried at 65 ^{o}C in an oven for 72 h to determine the dry weight (DW).

The whole-plant *water use efficiency (WUE)* measures the amount of carbon assimilated as biomass per unit of water used and was determined following the method described by [11].

The *Root shoot ratio* estimates crop root biomass and carbon input and was achieved by the method of [12]. Harvested plants were partitioned into root and shoot, washed thoroughly, and placed in well labelled paper bags to determine their respective weights. Then, oven-dried at 65 ^{o}C for 72 h to determine their dry weight and evaluate the root/shoot weight ratio.

Tolerance index (TI) was determined as the ratio among the biomass produced under drought stress and analogous biomass produced in well-watered conditions [13].

Determination of biochemical properties

Lipid peroxidation **(**Malondialdehyde (MDA); an index of lipid peroxidation) often used as a measure of oxidative stress during drought stress was isolated and determined using the method of [14]. The absorbance was measured at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA- complex of 1.56×10^5 $M^{-1}CM^{-1}$. *Proline* content was determined according to [15] based on proline's reaction with ninhydrin. The absorbance was recorded at 575 nm. The estimation of proline content for the six fonio genotypes studied was expressed as mg proline/g fresh weight and compared with the control group.

Catalase activity which converts Hydrogen peroxide (H_2O_2) to water molecule (H_2O) and Oxygen (O_2) was determined according to [16]. It was assayed calorimetrically at 620 nm and expressed as µmoles of H_2O_2 consumed/min/mg.

Ascorbate peroxidase (APX) activity which uses ascorbic acid as the electron donor to scavenge H_2O_2 was assayed following the method of [17] using the initial rate of decrease in ascorbate concentration. APX activity was determined as a decrease in ascorbate concentration, as measured by its absorbance wavelength at 290 nm over 30 s using Milton Roy Spectron 21D.

Glutathione reductase was assayed according to the method described by [18]. The absorbance was read at 412 nm.

The activity of *Superoxide dismutase* (SOD) was conducted following the method described by [19]. SOD activities were calculated by measuring the change in absorbance at 480 nm for 90 s at 30 s intervals.

Statistical analysis

Data generated were analyzed using IBM SPSS 26 (IBM Corp., Armonk, NY). Both analyses of variance (ANOVA) and T-test were used to

evaluate tolerance performance within the genotypes and between drought stress and control set ups. Mean differences were separated using Duncan Multiple Range Test and considered significant at $p < 0.01$.

Results and discussion

Physiological analysis of drought tolerance

At the early stage of drought stress, Figure 1 showed that WUE parameters for all evaluated genotypes subjected to the stress, were significantly ($p < 0.01$) reduced. Whereas genotypes P19, P22 and P23 showed no significant difference in their WUE at the late stage. Drought had significant effects on all fonio genotypes at the early stage. It could be concluded that genotypes P19, P22 and P23 performed in similar way with reference to WUE as a drought tolerant marker. Whereas the study of [20] reported that plants having the highest WUE are the most resistant to drought stress.

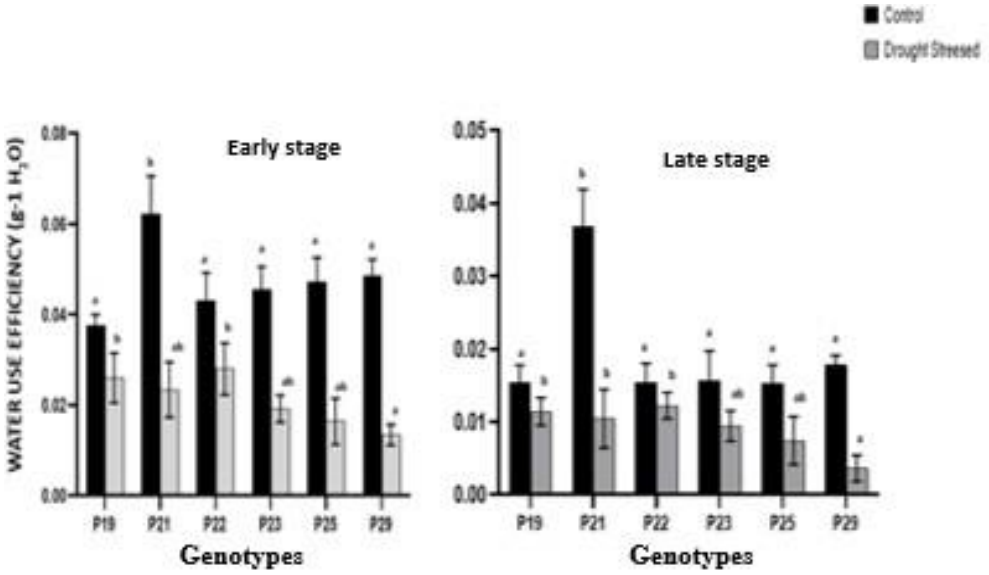

Fig. 1 Effects of drought on Water use efficiency of Drought Tolerant genotypes

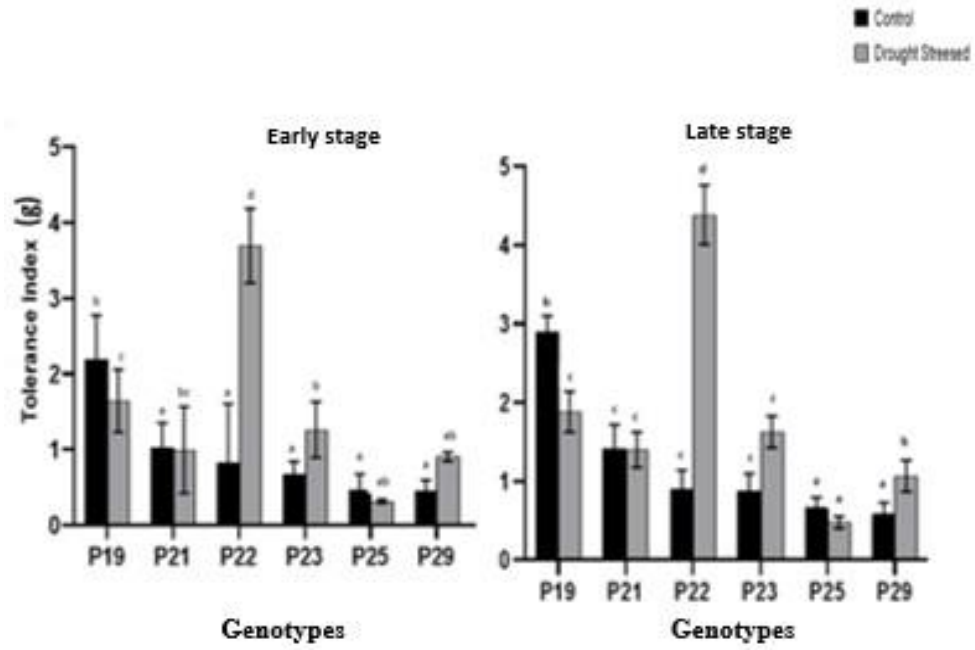

Fig. 2 Effects of drought on Tolerance index of Drought Tolerant genotypes

Among all the evaluated genotypes, only the T.I. of genotype P22 increased significantly at the early and late stages when compared to its control, as illustrated in Figure 2.The tolerance index (TI) is one of the best criteria to identify tolerant genotypes. Genotype P22 showed resilience to drought stress and its capability to adapt under water deficit conditions. This result confirmed that TI could be an effective drought tolerant marker for screening drought tolerant lines in fonio crop. The TI has also been reportedly used by [21] and [22] to screen and identify high drought tolerant genotypes under drought stress at the early and late stages.

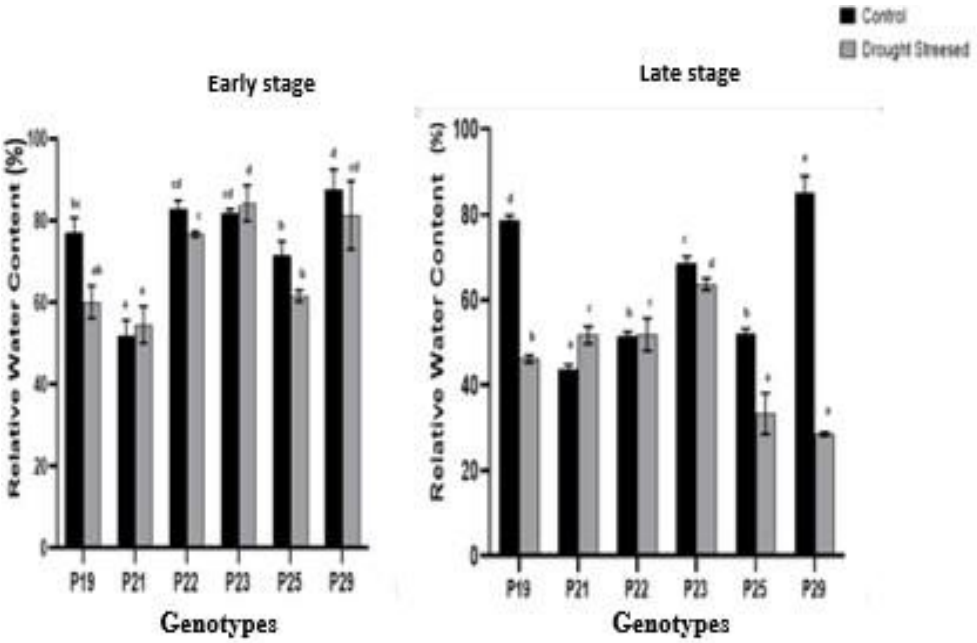

Fig. 3 Effects of drought on Leaf relative water content of Drought Tolerant genotypes

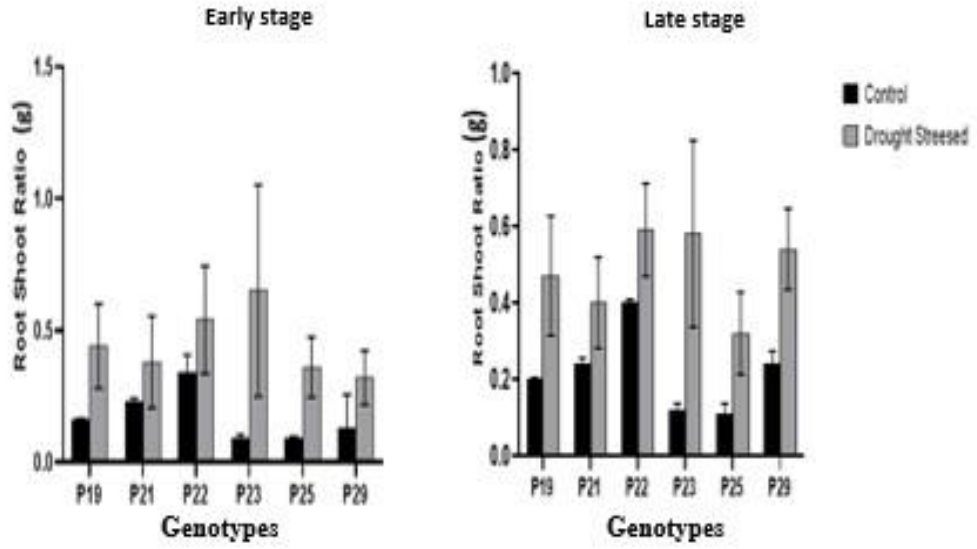

Fig. 4 Effects of drought on Root shoot ratio of Drought Tolerant genotypes

Regarding the LRWC, genotypes P19 and P25 at the early and late stage were significantly reduced under drought stress, while P21 remain statistically similar at the early stage and later increased significantly (Figure 3). The consistent LRWC increase of genotypes P21 under drought stress when compared to its control at the early and late stages, can be a result of turgidity that maintains stomatal opening and photosynthetic rates. The LRWC could also be an indicative drought tolerant marker in fonio crop. This corroborates the study of [23] on some wheat cultivars, that genotypes with higher LRWC are more drought tolerant than those with lower LRWC.

Though there was an increase in the RSR for all the evaluated genotypes under drought stress at the early and late stage, the increase was not significantly important (Figure 4). This non-significant increase depicts that shoot dry weight was affected owing to water loss through the stomata because of sun radiation as compared to the root system. This shows that the proportion of dry matter allocated to shoots was decreased compared to the roots. This difference indicates that protection mechanisms might be switched on to deal with plant water stress in fonio and rectify the amount of dry matter allocated to roots. The response of the RSR of fonio genotypes under drought stress also correlates with the work of [24], who reported an increase in the RSR of maize (*Zea mays*) when subjected to drought stress and [25] who reported an increase in the RSR of some bean genotype when subjected to drought stress.

Biochemical analysis of drought tolerance genotypes

Results in Table 2 revealed that, out of all the evaluated genotypes, only P19 (6.66 mg/g) and P23 (6.37 mg/g), under drought stress at early and late stage, showed consistent significant increase in proline properties, while the remaining genotypes had their proline reduced. The high accumulation of proline in P19 and P23 genotypes resulted in drought stress avoidance. Similar increase in proline response to drought stresses were observed in *Oryza sativa* [26], a close relative of fonio. It has been studied that the accumulation of proline in response to drought and salinity stress helps to facilitate the absorption of water [27].

Table 3 showed an increase of Lipid peroxidase content in P19, which is significantly different when compared to the control group at both stages. The significant change in P19 was evident that, drought treatment could lead to oxidative stress in fonio. However, membrane lipid peroxidation caused by drought stress was restored at the late stage in the remaining genotypes. In addition, drought at this late stage, in drought tolerant fonio could enhance adaptability to water stress at later growth stage. Therefore, the low lipid peroxidation content recorded at the late stage could be used to select tolerant genotypes as several authors on *Eleusine coracana* [28]**;** *Oryza sativa* [29] selected best tolerant varieties using low lipid peroxidation content.

Table 2.

Activities of Proline under drought conditions at early and late stage of drought stress on drought tolerant genotypes

	EARLY STAGE			LATE STAGE		
Genotype	**Control (mg/g)**	**Drought stress (mg/g)**	**p-value**	**Control (mg/g)**	**Drought stress (mg/g)**	**p-value**
P19	0.45 ± 0.06 a	3.80 ± 0.13 d	0.00	2.26 ± 0.08 a	6.66 ± 0.08 f	0.00
P21	2.57 ± 0.04 b	0.23 ± 0.10 a	0.00	4.31 ± 0.13 b	0.63 ± 0.19 a	0.00
P22	4.05 ± 0.07 c	2.00 ± 0.11 b	0.00	4.86 ± 0.16 c	3.20 ± 0.11 b	0.00
P23	0.53 ± 0.07 a	2.69 ± 0.14 c	0.00	2.37 ± 0.23 a	6.37 ± 0.08 e	0.00
P25	5.92 ± 0.02 d	4.94 ± 0.34 e	0.01	5.98 ± 0.09 d	5.41 ± 0.28 d	0.03
P29	4.12 ± 0.15 c	3.85 ± 0.09 d	0.06	4.54 ± 0.12 b	4.42 ± 0.09 c	0.24
	F=2214.30	F=271.08		F=310.16	F=627.66	

Table 3.
Activities of Lipid peroxidation content under drought conditions at early and late stage on drought tolerant genotypes

	EARLY STAGE			LATE STAGE		
Genotype	**Control (µmol/ml)**	**Drought stress (µmol/ml)**	**p-value**	**Control (µmol/ml)**	**Drought stress (µmol/ml)**	**p-value**
P19	0.34 ± 0.06 a	0.67 ± 0.06 ab	0.00	0.58 ± 0.05 a	1.04 ± 0.13 a	0.00
P21	0.51 ± 0.05 b	0.79 ± 0.04 c	0.00	1.53 ± 0.08 b	1.68 ± 0.09 b	0.10
P22	0.53 ± 0.04 b	0.75 ± 0.05b c	0.00	0.54 ± 0.17 a	0.76 ± 0.31 a	0.34
P23	0.39 ± 0.03 a	0.64 ± 0.06 a	0.00	0.42 ± 0.13 a	0.66 ± 0.30 a	0.27
P25	0.50 ± 0.02 b	0.90 ± 0.03 d	0.00	0.54 ± 0.11 a	0.81 ± 0.28 a	0.20
P29	0.47 ± 0.06 b	0.63 ± 0.07 a	0.04	0.49 ± 0.08 a	0.74 ± 0.27 a	0.20
	F=8.12	F=11.44		F=42.31	F=7.20	

Table 4.
Activities of Super Oxide Dismutase under drought conditions at early and late stage on drought tolerant genotypes

	EARLY STAGE			LATE STAGE		
Genotype	**Control (µmol/ml/min)**	**Drought Stress (µmol/ml/min)**	**p-value**	**Control (µmol/ml/min)**	**Drought Stress (µmol/ml/min)**	**p-value**
P19	2636.81 ± 93.90 d	1162.93 ± 75.40 b	0.00	907.96 ± 107.72 c	565.92 ± 190.57	0.05
P21	1256.22 ± 59.97 c	1200.25 ± 270.58 bc	0.74	373.13 ± 122.34 ab	808.46 ± 28.50	0.00
P22	914.18 ± 130.60 b	684.08 ± 137.52 a	0.10	329.60 ± 150.80 ab	652.98 ± 615.11	0.43
P23	1411.69 ± 119.95 c	659.20 ± 65.52 a	0.00	659.20 ± 225.69 c	628.11 ± 197.74	0.87
P25	708.95 ± 81.32 ab	1449.01 ± 21.54 c	0.00	105.72 ± 38.83 a	1057.21 ± 233.27	0.00
P29	572.14 ± 216.24 a	1019.90 ± 156.47 b	0.04	391.79 ± 161.57 b	1007.46 ± 300.25	0.04
	F=104.21, p < 0.01	F=13.59, p < 0.01		F=11.12, p < 0.01	F=1.29, p=0.33	

The activities of SOD at the early stage decreased significantly in P19 and P23 under drought stress condition when compared to their respective controls. However, it increased significantly in double folds for P25 (1449.01 µmol/ml/min) and P29 (1019.90 µmol/ml/min) genotypes at early stage and significantly higher at the late stage (Table 4). Genotype P21 has an increased activity of SOD at the later stage to counter the deleterious effects of superoxide radicals released from the significant increase in lipid peroxidation content at the early stage. A similar occurrence has been recorded in other crops; Oilseed Rape (*Brassica napus*) [30]; and tomato seedlings [31] that reacted with increased lipid peroxidation content and increased their SOD activities as an enzymatic defense system against drought-induced oxidative stress. Despite the increase in lipid peroxidation content of genotype P19, SOD activities decreased under drought conditions. This corroborates with the works on bent grass (*Agrostis spp*.) [9] exposed to drought. Decreased activity of SOD indicates that the ability to scavenge for superoxide radicals was weakened by drought stress, which may result in free radical- mediated damage, including lipid peroxidation in membranes [32]. This implies that drought stress had no effects on SOD in some plants.

The results on table 5 showed a consistently significant decrease in CAT activities for genotypes P21, P22, and P23 at the early and late stage while the remaining genotypes behave differently. The reduction in CAT activities in genotypes P21, P22 and P23 may have led to the increase in Lipid peroxidation content recorded at the early and late stage. An increase in Lipid peroxidation content along with reduced CAT activities in two cultivars of Kentucky grass when exposed to drought stress treatment was also

reported in the study of [33]. Despite the significant increase in Lipid peroxidation content of P19 recorded at the early and late stage, the observed decrease in Superoxide SOD and CAT activities at both stage showed the inability of these two enzymes to prevent or decrease the deleterious effects of ROS in the genotype.

There was also a consistently significant decrease in GR activities for genotypes in P22, P23, P25 and P29 at the early and late stage under drought stress conditions when compared to their respective control. While genotypes P19 showed a statistically similar performance (Table 6). The reduced GR activities observed in genotypes P22, P23, P25 and P29 could not contribute to better recuperative effects of high lipid peroxidation content recorded in this study. This could be attributed to the reduced NADPH availability since drought stress usually results in a decrease in the supply of reductants such as ATP and NADPH. It is also possible that Reactive Oxygen Species (ROS) accumulation resulted in the inhibition of enzymes involved in the ROS-scavenging system [34].

Activities of APX during drought treatment at early and late stage decreased significantly in P19 and P21 when compared to their respective control, while the activities of APX in genotypes P22 and P29 remain statistically similar for both control and drought stressed plants (Table 7). The reduction in APX activities recorded in genotypes P19 and P21 coupled with the decreased level of CAT activities observed in the study may have led to increased level of Lipid peroxidation contents. The statistical similar performance as seen in genotypes P22 and P29 indicated that the non-significant lipid peroxidation content observed for both genotypes at the late stage was mainly associated with APX enzymes scavenging for H_2O_2. A similar occurrence was recorded in the study of [9] on Velvet bent grass (*Agrostis canina* L.) where no differences in APX activity was observed among drought stress treatments at day 1 and 7.

Table 5.

Activities of Catalase under drought conditions at early and late stage on drought tolerant genotypes

	EARLY STAGE			LATE STAGE		
Genotype	control (µmol/ml/min)	Drought stress (µmol/ml/min)	P value	Control (µmol/ml/min)	Drought stress (µmol/ml/min)	P value
P19	549.26 ± 6.35 a	493.16 ± 5.23 b	0.06	529.18 ± 1.44 ab	512.55 ± 6.35 b	0.02
P21	580.43 ± 5.23 b	491.08 ± 5.23 b	0.03	556.88 ± 11.57 c	520.87 ± 8.40 b	0.01
P22	545.80 ± 9.37 a	520.17 ± 4.33 c	0.04	528.48 ± 3.17 ab	484.85 ± 4.33 a	0.00
P23	572.81 ± 4.33 b	565.19 ± 4.16 d	0.00	539.57 ± 7.30 bc	513.94 ± 8.40 b	0.00
P25	597.75 ± 7.30 c	500.78 ± 5.50 b	0.54	589.44 ± 20.18 d	585.97 ± 5.50 c	0.00
P29	574.89 ± 12.53 b	473.77 ± 9.06 a	0.00	510.48 ± 4.33 a	482.08 ± 8.32 a	0.31
	F=18.05, p < 0.01	F=91.31, p < 0.01		F=18.22, p < 0.01	F=84.82, p <0 .01	

Table 6.

Activities of Glutathione reductase under drought conditions at early and late stage on drought tolerant genotypes

	EARLY STAGE			LATE STAGE		
Genotype	Control (µmol/ml)	Drought treated (µmol/ml)	P value	Control (µmol/ml)	Drought treated (µmol/ml)	P value
P19	28.88 ± 2.59 b	25.61 ± 0.64 c	0.101	31.56 ± 1.32 a	31.21 ± 0.51 c	0.686
P21	29.06 ± 0.34 b	27.36 ± 0.99 cd	0.048	31.97 ± 1.74 a	32.60 ± 0.81 d	0.602
P22	33.49 ± 0.95 c	20.50 ± 0.47 b	0.000	37.52 ± 0.60 c	31.65 ± 0.47 cd	0.000
P23	19.84 ± 0.61 a	15.09 ± 0.41 a	0.000	34.52 ± 0.62 b	15.62 ± 1.12 a	0.000
P25	36.35 ± 0.47 d	26.42 ± 0.89 cd	0.000	41.86 ± 0.56 d	35.28 ± 0.34 e	0.000
P29	39.71 ± 0.47 e	28.47 ± 2.93 d	0.003	40.34 ± 1.48 d	29.55 ± 0.74 b	0.000
	F=102.41	F=46.46		F=41.73	F=285.03	

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Table 7.

Activities of Ascorbate peroxidase under drought conditions at early and late stage on drought tolerant genotypes

	EARLY STAGE			LATE STAGE		
Genotype	**Control**	**Drought stress**	**P value**	**Control**	**Drought stress**	**P value**
P19	0.07 ± 0.00 e	0.01 ± 0.00 a	0.000	0.06 ± 0.00 e	0.03 ± 0.00 c	0.000
P21	0.06 ± 0.00 d	0.02 ± 0.00 b	0.000	0.03 ± 0.00 c	0.02 ± 0.00 bc	0.005
P22	0.01 ± 0.00 a	0.01 ± 0.00 a	0.275	0.02 ± 0.00 ab	0.02 ± 0.01 bc	0.829
P23	0.04 ± 0.01 c	0.04 ± 0.00 c	0.107	0.05 ± 0.01 d	0.03 ± 0.00 c	0.002
P25	0.03 ± 0.00 b	0.01 ± 0.01 a	0.021	0.02 ± 0.00 ab	0.02 ± 0.00 bc	0.018
P29	0.01 ± 0.00 a	0.01 ± 0.00 a	0.859	0.01 ± 0.00 a	0.01 ± 0.00 a	0.292
	F=138.75	23.88		F = 78.00	F=10.81	

**$p < 0.01$; *$p < 0.05$; vertical values (mean ± SD) with the same alphabets are not significantly different at $p < 0.05$ using Duncan Multiple Range Test

Table 8.

Interaction effects of genotypes, treatment and time on oxidative enzymes

Source of variation (Sov)	df	SOD Activity	CAT Activity	GR Activity	APX Activity
Genotypes	5	647429.135**	5575.486**	283.809**	0.002**
Treatment	1	49313.178ns	1535.192**	918.949**	0.004**
Time	1	4786124.075**	35187.032**	494.464**	0.000ns
Genotype x treatment	5	1198598.616**	2544.408**	69.259**	0.001**
Genotype x time	5	401067.634**	1028.435**	22.054**	0.00019**
Treatment x time	1	1342627.878**	6217.815**	0.505ns	0.000401**
Genotype x treatment x time	5	94785.575ns	1619.099**	41.325**	0.000218**
Error	48	39637.026	68.364	1.283	0.000

**$p < 0.01$; *$p < 0.05$

Effects of genotypes, treatment and time on antioxidant enzymes

The effects of drought on evaluated fonio genotypes confirmed that the varying increase and decrease of antioxidant defense capacity (SOD, CAT, GR and APX) during drought were dependent on plant genotype, interaction of genotype x treatment and the interaction effects of Treatment x time (Table 8). This study showed a significantly enhanced SOD activity in fonio genotypes when exposed to longer time treatment. SOD processing is known to be substrate inducible, an increase in the SOD activity may be attributed to increased production of reactive oxygen species (Superoxide radicals) O_2 as substrate that led to significant increase in SOD activity of fonio genotypes as compared to other enzymes activities [35]. This is consistent with other studies reporting the increased SOD activity in response to duration of drought stress in sunflower [36].

Conclusions

All the evaluated genotypes in the study behaved differently under drought stress conditions. The discrepancies observed in antioxidant activities may be attributed to the duration of drought as well as important protective roles played by each antioxidant enzymes for survival of severe water deficit. Genotype P25 showed an effective adaptation of water deficit under drought stress

condition, the super oxide radicals produced because of drought stress has been converted to hydrogen peroxide by the increased SOD level observed at the early and late stage. The hydrogen peroxide produced has been converted to oxygen and water by the enhanced CAT activities which was like the control at the early stage but reduced at the late stage owing to the stability observed in the lipid peroxidase content when compared to the control. Other genotypes responded with different enzymatic defense. Drought stress was accompanied by slight morphological changes in some genotypes during drought stress which led to the variation observed in the physiological study. This change can be attributed to some increased activity or adaptive features in the genotypes to combat drought stress.

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