Impact of Fish Guano and Abscisic Acid on Physiological Traits of Grain Amaranth (*Amaranthuscruentus* L.) under Moisture Stress

ABSTRACT: Field trials were conducted during the 2021 and 2022 at the Teaching and Research Farm of the Facultyof Agriculture Bayero University Kano (110 97′ 98.6″N 80 42′03.7′″E) and Research Farm of Aliko DangoteUniversityofScienceandTechnologyWudil(11̊25′NandLong9̊E)todeterminethe impactoffishguano(FG)and abscisic acid(ABA)leaf area index (LAI), stomatal conductance(SC), photosynthetic active radiation (PAR)andrelativewatercontent(RWC)ofgrainamaranthundermoisturestressconditions.Treatmentsconsistedofmoisture stress stages (vegetative, flowering and grain filling), FG (0, 0.1 and 0.2kg) and ABA concentrations (0, 20and 50 µmolL^{-1}) and laid out in a split-plot design and replicated three times. Moisture stress was applied in the main plots, while FG and ABA treatments were applied in the subplots.Data was collected on LAI, SC, PAR and RWC and subjected to analysis of variance(ANOVA) using GENSTAT 17th edition software. Significant treatment means were separated using the StudentNewman KeulsTest (SNK) at 5% probability level. In 2021 and 2022, the LAI and RWC of the plants at BUK and Wudil were significantly ($p<0.001$) boosted by the application of 0.2 kg FG levels and 50 µmolL^{-1} ABA. In the 2021 season, plants experiencing moisture stress during flowering exhibited significantly ($p<0.05$) higher RWC at BUK. Additionally, the application of 20 µmolL^{-1}ABA had a significant ($p<0.05$) effect on stomatal conductance at BUK and Wudil in both seasons. The results of this study suggested that FG and ABA could be useful strategies for improving the performance and resilience of grain amaranth crops in the face of moisture stress.

Key words: Fish Guano, Grain amaranth,pseudocereal

1 Introduction

Grain amaranth is a pseudocereal that has been widely studied and associated with functional properties andattractive medical benefits (Nataly *et al.*,2023). Amaranth seeds contain more protein (17.6%) per 100% than other crops like corn and rice and contain several essential amino acids (Soriano *et al.*, 2018). It is high in fibre and low insaturated fat (DPP 2010). Greer and Driver (2000) reported that some aquatic organisms such as algae and fish wastehadnutritionalcontentssimilartomineralfertilizer exploited ingreenhousecultivation.ABA,anisoprenoid phytohormone, regulates various physiological processes, including stomatal opening and protein storage,and provides adaptation to many plants' stresses (Sah*et al.*, 2016). There is little information on the role of increasedfertilizationrateandABAconcentrationsinmediatingplantresponsestowaterstress,eventhoughABAisknownto beageneralgrowthinhibitorduring germinationand seedling establishment(Maria *et al.*,2014).ExperimentsontheeffectsofwaterstressandABAtreatmenton leaf morphology and floral development in spinach were undertaken to investigate ABA's role in water stress'sdevelopmental consequences and this suggests that ABA may have an essential role in determining the pattern ofplant development, at least in the presence of water stress. The application of fish guano can provide readilyavailablenutrientstosupporttheplant'sphysiologicalprocessesundermoisturestressconditions,whentheuptakeof nutrients can be challenging due to reduced root activity. Exogenously applying ABA can enhance the plant'sabilitytocopewithmoisturestressbytriggeringvariousphysiologicalandbiochemicalchanges whichcanultimately improve the plant's resilience and growth under water-limited conditions. This study was therefore carriedout to examine the effects of fish guano and ABA on LAI, SC, PAR and RWC of grain amaranth under moisturestress conditions.

2 Materials and Methods

2.1 Experimental sites

The experiment was conducted during the 2021 and 2022 dry seasons at Teaching and Research Farm Faculty ofAgricultureBayero University Kano and Aliko Dangote University of Science and Technology Farm Wudil, Kano State.

2.2 Treatments and Experimental design

Treatments consisted of induced moisture stress at (vegetative, flowering and grain filling), FG (0, 0.1 and 0.2kg)and abscisic acid concentration (0, 20 and 50µmolL^{-1}) and laid out in a split plot design with three replications.Induced moisture stress was assigned to the main plot while FG and ABA were assigned to the sub plot.

2.3 Preparation and application of materials

Fish guano powder 200g was suspended in 4 Liter of distilled water. It was mixed thoroughly, and serially diluted totheprescribedratesandsprayedacrossseedlingsusingthehand-heldwateringcan(Angibaud,DeromeandSpecialties2016).Astocksolutionwaspreparedbymixing100mgABApowderin 1mlethanol.Aweighedquantity of ABA (as per treatment) was added in a graduated cylinder and 1 L volume was made in a volumetricflask by adding distilled water. Solutions were immediately foliage applied with a hand sprayer (PhytoTechnology,Laboratories 2016).

2.4 Cultural practices

2.4.1 Land preparation: The experimental sites were cleared manually, ploughed and harrowed to obtain a finetilt soil. The site was divided into gross plots size of 3 x 4.5 m (13.5 m^2) made up of 6 rows each and net plot area of 3.0 m^2. A spacing of 1.5m between replicationsand 1.0 m between blocks was left as an alley. Discard between sub-plots was 0.5m.

2.4.2 Sowing: Seeds were sown in mid-January 2021 on a prepared nursery bed for four weeks by drilling method. Seedswere covered with soil and mulched to speed up the germination process.

2.4.3 Transplanting: Seedlingsweretransplantedtothefieldmanually,10seedlingsweretransplantedoneachof the 6 rows at a depth of 2.0 cm with a spacing of 75 cm x 30 cm between stands and rows, respectively.

2.4.4 Weeding: Weeding was done manually using a hoe

2.4.5 Pesticidesapplication: Garlicextractswereappliedthricetomitigatetheinfectionofleafminer *Liriomyzahuidobrensis*.

2.4.6 Harvesting: TheAmaranthgrainswereharvestedfromthenetplotarea byuprootingusingahoeat12WAT.Threshi ng was done manually.

2.5 Data Collection

Data were collected at the vegetative stage, flowering stage and grain filling which coincide with 4, 8 and 12 weeks aftertransplanting (WAT) on 5 tagged plants from the two inner rows (net plot). Data was collected using standard agronomic procedures on:

2.5.1 Leafareaindex: TheLAIwasdeterminedusingtheAccuPARmodelLP-80PAR/LAICeptometer(Decagon Devices, INC. Pullman, USA).

2.5.2 Photosyntheticallyactiveradiation: ThiswasdeterminedbyusingECA-PB0402Photosyntheticapparatus Beijing Yikangnong science and technology development CO. LTD.

2.5.3 Stomatal conductance: This was measured to know the functions of a leaf using a leaf Porometer AP4Delta devices-Cambridge- U.K.

2.5.4 Relative water content (%): The RWC was measured as described by Hayat *et al.* (2007). Five freshleaves from tagged plants (two cm diameter, excluding midrib) were taken, weighed and immediately floated ondeionizedwaterin Petri dishes.EachPetri dishwassuppliedwith100mldeionizedwatertosaturatetheleafdiscfor24h,underdarkconditions.At24h,theleafdiscswerer emovedfromthewaterandblottedwithtissuepaperstoremovetheadheringwaterandtheturgormasswasrecorded.Theleafdis cswerethendriedat70°Cfor48h.The RWC was calculated as follows:

Where, FW = fresh leaf weight/mass; DW = dry leaf weight and TW = turgor weight of leaf

2.6 Data Analysis

Data collected were subjected to analysis of variance (ANOVA) using Genstat 17th edition. Significant treatmentmeans were separated using the Student Newman-Keuls test at 5% level of probability.

3 Results

3.1 Leaf Area Index

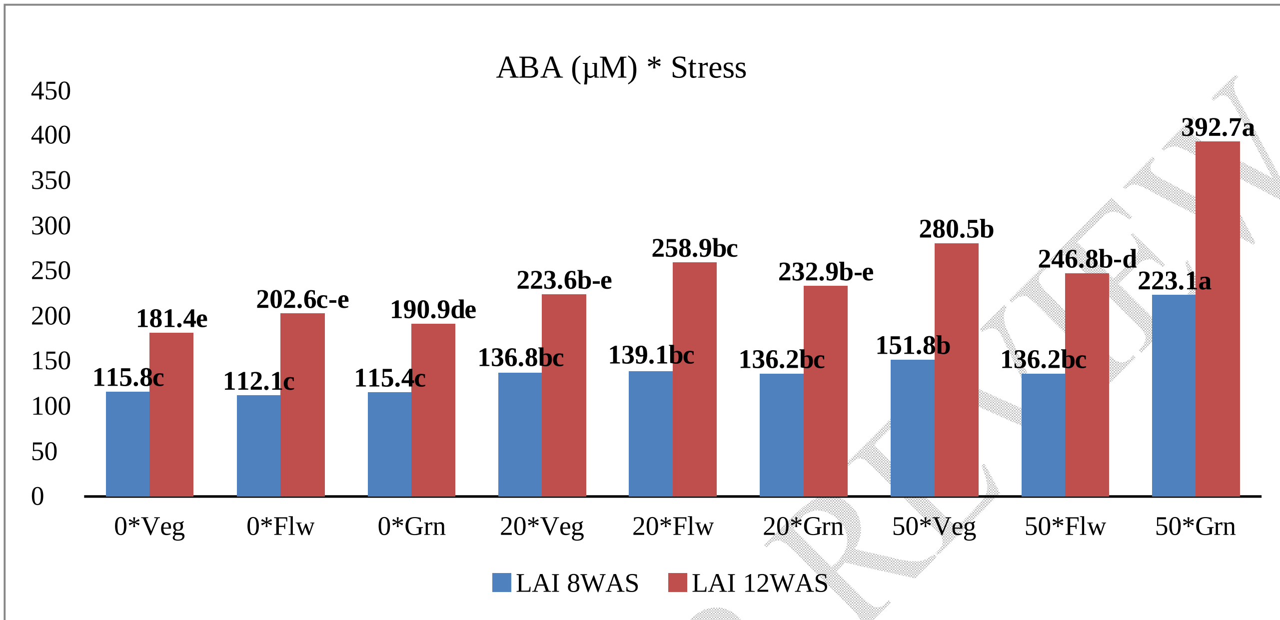
The effect of fish guano and ABA on LAI of grain amaranth under stress condition at BUK and Wudil in 2021 and2022 dry season is presented in Table 1. There were no significant ($p>0.05$) differences in vegetative, flowering andgrain filled at BUK and Wudil in 2021 and 2022. No significant ($p>0.05$) differences were recorded for LAI ofamaranth for FG at BUK in 2021 and 2022. However, there was significant difference ($p<0.05$) in LAI at 4 and8WATatWudilin2022.At4WAT,thehighest(113.8) LAI was obtained at 0.1kgFG,followedby0kg(90.5)while least was recorded at 0.1kg FG (87.2). Similar trend was also observed at 8WAT (Table 1). The result of ABAconcentration on LAI of grain amaranth showed significant difference at BUK in 2021. In 2022, at BUK, the LAIresult indicated that there was no significant ($p>0.05$) increases at 4WAT and 12WAT while at 8WAT there wassignificant ($p<0.05$) increases with increase in the level of ABA with the highest LAI of 166.3 at 50μm ABA. In2021, at Wudil, there was significant ($p<0.05$) increase with increase in the dosage of ABA at 4, 8 and 12WAT. Thehighest LAI recorded for 50μm ABA were 81.5, 117.3 and 157.8 at 4, 8 and 12WAT respectively. The result further showed that there were significant ($p<0.05$) increase of LAI in 2022 at Wudil at 4, 8 and 12WAT. Thehighest (227.2) LAI was obtainedwith 50μm ABA at 12WAT while the least (83.9) was recorded at 0μm ABA at4WAT at Wudil in 2022. There was significant interaction of stress x ABA at 8 and 12 WAT and fish guano x ABAat4,8and12WATin2022atWudil(Table1).At12WAT,therewassignificant($p<0.05$)interactiononLAIat both vegetative to grain filled stage where the highest (275.5) LAI interaction was equally recorded at 50µm ABA atvegetative stage while least (139.4) was recorded at vegetative stage at 0µm ABA in 12WAT Wudil 2022 dryseason.

Table 1: Effect of Fish Guano and Abscisic Acid Concentrations on Leaf Area Index per plant of `grain Amaranthunder moisture stress conditions at BUK and Wudil during the 2021 and 2022 dry seasons

	BUK						Wudil					
	2021			2022			2021			2022		
	Weeks after transplanting (WAT)											
Treatments	4	8	12	4	8	12	4	8	12	4	8	12
Stress (S)												
Vegetative	76.2	94.0	114.1	154.0	153.3	218.0	64.7	100.0	130.1	96.7	133.5	178.0
Flowering	78.1	102.0	118.8	204.0	143.8	245.0	68.6	99.0	129.8	81.4	132.7	170.9
Grain Filling	84.1	136.0	116.1	104.0	126.7	195.0	67.9	101.0	129.7	113.4	156.0	196.8
p-value	0.351	0.545	0.869	0.438	0.539	0.568	0.826	0.954	0.999	0.070	0.225	0.593
SE±	3.55	26.5	6.25	49.3	15.82	30.9	4.60	4.48	6.82	6.79	8.92	17.32
Fish Guano (F) (kg)												
0	75.8	100.0	114.9	237.0	142.0	191.0	64.2	100.5	130.3	90.5^{b}	134.8^{b}	169.3
0.1	80.4	100.0	116.7	109.0	141.9	221.0	65.6	97.8	125.4	87.2^{b}	129.1^{b}	174.9
0.2	82.2	132.0	117.4	115.0	140.0	246.0	71.5	101.7	134.0	113.8^{a}	158.3^{a}	201.6
p-value	0.280	0.465	0.874	0.235	0.989	0.343	0.271	0.672	0.250	0.008	0.011	0.053
SE±	2.86	20.6	3.49	59.0	10.63	26.1	3.33	3.15	3.61	6.27	6.96	9.77
ABA (A) (µM)												
0	64.9^{c}	78.0^{b}	90.8^{c}	237.0	107.4^{b}	186.0	50.4^{c}	77.3^{c}	98.8^{c}	83.9^{b}	114.4^{c}	141.9^{c}
20	80.1^{b}	100.8^{ab}	118.6^{b}	109.0	150.2^{a}	221.0	69.4^{b}	105.5^{b}	133.0^{b}	94.1^{b}	137.4^{b}	176.6^{b}
50	93.5^{a}	153.1^{a}	139.5^{a}	115.0	166.3^{a}	253.0	81.5^{a}	117.3^{a}	157.8^{a}	113.6^{a}	170.4^{a}	227.2^{a}
p-value	<.001	0.039	<.001	0.394	<.001	0.203	<.001	<.001	<.001	0.006	<.001	<.001
SE±	2.86	20.6	3.49	59.0	10.63	26.1	3.33	3.15	3.61	6.27	6.96	9.77
Interaction												
S*F	0.340	0.408	0.638	0.747	0.923	0.459	0.784	0.700	0.631	0.865	0.393	0.184
S*A	0.292	0.481	0.105	0.275	0.817	0.665	0.777	0.265	0.726	0.175	0.007	0.048
F*A	0.574	0.303	0.234	0.726	0.711	0.286	0.244	0.142	0.163	0.039	0.002	0.004
S*F*A	0.364	0.633	0.297	0.318	0.196	0.479	0.976	0.924	0.858	0.973	0.710	0.707

Means followed by the same letter(s) in a column within a treatment group are not significantly different at a 5% level probability using SNK

Figure1:InteractionofStressxAbscisicAcidonLeafAreaIndexofgrainAmaranthat8and12WATatWudilduring 2022 dry season



Means followed by same letter in a column within a treatment group are not significantly different at 5% level probability using SNK

3.2 Photosynthetic active radiation (PAR)

The result of FG and ABA concentrations on PAR of grain amaranth under moisture stress conditions at BUK andWudil during the 2021 and 2022 dry season are presented in Table 2. The stressed group revealed that at BUK in2021 season, there were no significant (p>0.05) differences of PAR at 4, 8 and 12WAT while in 2022 season, theresult showed significant (p<0.05) difference at 4 and 12WAT on PAR of grain amaranth. It was recorded that 637.1wasthehighestPARobtainedatgrainfillingconditionat4WATwith444.9asthelowestPARrecordedatflowering condition. At 12WAT, in 2021 season at BUK, 44.50 was the highest PAR value while 6.93 was thelowest value recorded. At Wudil, the result showed clearly that there were no significant (p>0.05) differences at12WAT across the 2021 and 2022 seasons. The FG group indicated that from 4 to 12WAT at BUK 2021 season,there were no significant (p>0.05) differences. Similar, with the exception (p<0.05) of 8WAT, there were nostatistical (p>0.05) differences. At 8WAT, grain amaranth gave highest PAR value at 0kg FG (35.95)followed by 0.1kg (34.67) while lowest PAR value were recorded at 0.2kg fish guano concentration level. The effectof FG on PAR at Wudil in both 2021 and 2022 seasons revealed that there were no significant (p>0.05)differencesingrainamaranthPARacross4,8and12WAT.InthisstudyitwasclearlyshownthatABA concentrationlevelhadnosignificant(p>0.05)responsesongrainamaranthPARatBUKandWudilinboth2021and 2022 dry season despite all numerical variations.

Table2:EffectofFishGuanoandAbscisicAcidConcentrationsonPhotosyntheticActiveRadiation(µmols^{-1})perplant of grain amaranth under moisture stress conditions at BUK and Wudil during the 2021 and 2022 dry seasons

	BUK						Wudil					
	2021			2022			2021			2022		
	Weeks after transplanting (WAT)											
Treatments	4	8	12	4	8	12	4	8	12	4	8	12
Stress (S)												
Vegetative	0.596	0.458	0.612	575.7^{a}	36.09	44.50^{a}	495.0	467.0	539.0	324.0	562.0	525.0
Flowering	0.478	0.305	0.467	444.9^{b}	34.31	6.93^{b}	714.0	502.0	521.0	232.0	522.0	535.0
Grain Filling	0.649	0.405	0.490	637.1^{a}	33.75	43.61^{a}	578.0	553.0	556.0	328.0	529.0	483.0
p-value	0.327	0.560	0.112	0.008	0.327	<.001	0.304	0.642	0.729	0.591	0.702	0.554
SE±	71.7	94.8	39.2	21.90	0.996	1.806	86.4	61.5	29.3	69.7	34.4	33.4
Fish Guano (F) (kg)												
0	0.551	0.354	0.488	569.1	35.95^{a}	32.26	519.0	515.0	452.0	246.0	537.0	517.0
0.1	0.663	0.348	0.536	527.7	34.67^{ab}	31.81	544.0	401.0	608.0	312.0	566.0	540.0
0.2	0.509	0.465	0.544	560.8	33.53^{b}	30.97	724.0	606.0	556.0	326.0	511.0	486.0
p-value	0.222	0.340	0.741	0.144	0.032	0.710	0.103	0.099	0.280	0.569	0.241	0.138
SE±	64.0	62.9	55.2	15.41	0.628	1.112	72.4	66.0	69.2	56.1	22.8	19.2
ABA (A) (µM)												
0	0.599	0.369	0.488	556.6	34.53	31.97	574.0	484.0	570.0	313.0	553.0	520.0
20	0.581	0.448	0.562	525.8	35.18	31.46	558.0	541.0	583.0	321.0	522.0	509.0
50	0.543	0.351	0.518	575.2	34.44	31.61	655.0	497.0	463.0	250.0	539.0	514.0
p-value	0.824	0.518	0.642	0.083	0.661	0.945	0.597	0.814	0.411	0.624	0.629	0.923
SE±	64.0	62.9	55.2	15.41	0.628	1.112	72.4	66.0	69.2	56.1	22.8	19.2
Interaction												
S*F	0.224	0.716	0.088	0.155	0.994	0.738	0.033	0.258	0.547	0.353	0.786	0.474
S*A	0.127	0.991	0.125	0.091	0.983	0.858	0.610	0.714	0.823	0.398	0.964	0.505
F*A	0.798	0.456	0.500	<.001	0.418	0.537	0.834	0.327	0.650	0.240	0.948	0.771
S*F*A	0.174	0.369	0.549	0.254	0.840	0.065	0.326	0.768	0.570	0.607	0.967	0.415

Means followed by the same letter(s) in a column within a treatment group are not significantly different at a 5% level probability using SNK

3.3 Relative Water Content (RWC)

TheresultontheeffectofFGandABAconcentrationonRWCofgrainamaranthundermoisturestressconditionsat BUK and Wudilare presented in Table 3. The result on the effect of stress on RWC of grain amaranth indicatedthat there was significant ($p<0.05$) differences at 4 and 12WAT. At 12WAT highest RWC was obtained at floweringstage with 71.7%. There were no significant ($p>0.05$) differences of RWC at BUK in 2022 season. Moreover, therewere no significant ($p>0.05$) difference of RWC of grain amaranth at Wudil in 2021 and 2022 under different stresscondition. The result on RWC of grain amaranth in FG group revealed significant ($p<0.05$) differences at 4, 8 and12WATatBUKin2021.At4WAT,48.29%wasthehighestRWCat0.2kgfishguanowhile40.09wasthelowestat 0kg FG concentration level. There were linear increases of RWC with increase concentration levels of fish guanoat8and12WATwhere58.92and71.2%werethehighestRWCobtainedat0.2kgFGfor8and12WATrespectively.In2022 atBUK,theresultrevealedsignificant($p<0.05$)differenceat12WATwiththehighestRWCof 50.2% at 0.2kg fish guano concentration level. At Wudil, the result showed that fish guano has no significant($p>0.05$)effectonRWCofgrainamaranthat2021and2022.TheresultontheeffectofABAconcentrationonRWC of amaranth showed that with the exception of 12WAT ($p<0.05$) there were no significant ($p>0.05$) differenceat both BUK and Wudil in 2021 and 2022 season respectively. At 12 WAT, at BUK in 2022, the result indicated thehighest (70.5) RWC of grain amaranth. The result further revealed significant ($p<0.05$) interactions between FG andABA at 8 WAT at BUK in 2021 season. The effect of FG and ABA interaction on RWC at 8 WAT at BUK in 2021dry season is presented in Figure 4. The result indicated clearly that highest RWC (66.45) was recorded at 0.2kg FGx 50µm ABA followed by 57.00 at 0.2kg FG x 20µm ABA while least (47.39) was recorded at 0kg FG x 0µm ABA.

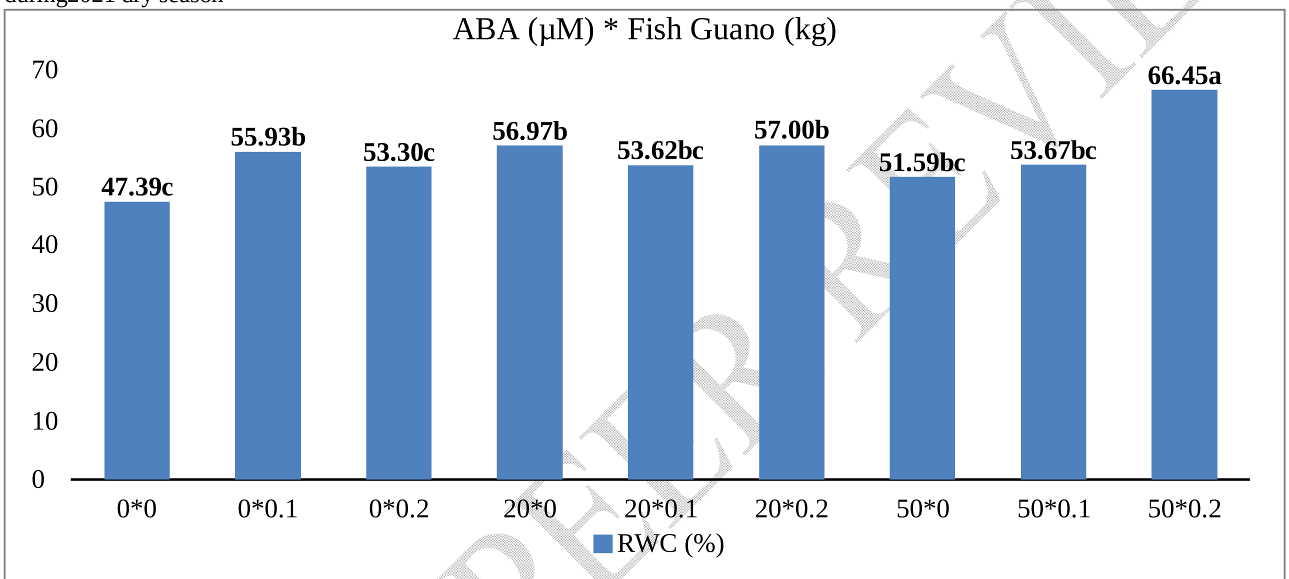
Table 3: Effect of Fish Guano and Abscisic Acid Concentrations on the Relative Water Content (%) per plant of grain amaranthunder moisture stress conditions at BUK and Wudil during the 2021 and 2022 dry seasons

	BUK						Wudil					
	2021			2022			2021			2022		
	Weeks after transplanting (WAT)											
Treatments	4	8	12	4	8	12	4	8	12	4	8	12
Stress (S)												
Vegetative	44.50[a]	52.90	65.7[b]	29.8	25.13	41.4	47.5	26.0	38.4	47.5	26.0	38.4
Flowering	47.15[a]	57.99	71.7[a]	33.3	27.40	47.6	57.0	24.5	34.9	57.0	24.5	34.9
Grain Filling	40.73[b]	54.41	64.4[b]	33.3	28.96	47.4	50.2	33.6	37.6	50.2	32.5	37.6
p-value	0.018	0.085	0.035	0.697	0.762	0.480	0.504	0.503	0.771	0.504	0.607	0.771
SE±	0.896	1.184	1.33	3.17	3.566	3.74	5.44	5.39	3.49	5.44	5.64	3.49
Fish Guano (F) (kg)												
0	40.09[c]	51.98[b]	61.9[b]	30.5	26.24	38.0[b]	50.8	25.6	35.5	50.8	25.6	35.5
0.1	44.00[b]	54.40[b]	68.7[a]	34.1	28.06	48.2[a]	50.5	29.7	39.1	50.5	28.6	39.1
0.2	48.29[a]	58.92[a]	71.2[a]	31.9	27.18	50.2[a]	53.3	28.7	36.3	53.3	28.7	36.3
p-value	<.001	0.008	0.002	0.396	0.733	0.039	0.814	0.540	0.459	0.814	0.679	0.459
SE±	1.362	1.511	1.76	1.85	1.630	3.50	3.38	2.68	2.10	3.38	2.78	2.10
ABA (A) (µM)												
0	41.89	52.20	64.2[b]	30.9	24.85	39.9	49.9	29.2	34.5	49.9	29.2	34.5
20	44.26	55.86	67.0[ab]	30.5	29.25	48.7	53.3	31.8	38.4	53.3	30.7	38.4
50	46.23	57.24	70.5[a]	35.1	27.38	48.0	51.5	23.0	38.0	51.5	23.0	38.0

p-value	0.089	0.061	0.049	0.161	0.170	0.159	0.767	0.068	0.364	0.767	0.129	0.364
SE±	1.362	1.511	1.76	1.85	1.630	3.50	3.38	2.68	2.10	3.38	2.78	2.10
Interaction												
S*F	0.881	0.718	0.948	0.225	0.810	0.748	0.949	0.149	0.430	0.949	0.268	0.430
S*A	0.131	0.147	0.188	0.093	0.773	0.568	0.898	0.594	0.672	0.898	0.794	0.672
F*A	0.226	0.011	0.145	0.779	0.083	0.673	0.614	0.436	0.945	0.614	0.405	0.945
S*F*A	0.916	0.975	0.500	0.590	0.861	0.995	0.590	0.906	0.307	0.590	0.925	0.307

Means followed by the same letter(s) in a column within a treatment group are not significantly different at a 5% level probability using SNK

Figure 2: Fish Guano x Abscisic Acid interaction on Relative Water Content (%) of grain Amaranth at 8WAT at BUK during2021 dry season



Means followed by same letter in a column within a treatment group are not significantly different at 5% level probability using SNK

3.4 Stomatal Conductance

The result on the effect of FG and ABA concentrations on stomatal conductance of grain amaranth under moisturestress conditions at BUK and Wudil during the 2021 and 2022 dry season is presented in Table 4. The stress groupindicated that there was significant ($p<0.05$) difference on stomatal conductance of grain amaranth at BUK 2021season at 4WAT and 12WAT. At 4WAT the result showed a linear increase of stomatal conductance with advancingof stress condition from vegetative to grain filled stage. At 2022 season in BUK the result however showedsignificant($p<0.05$)differencesat12WATwheregrainfillinghadaveryhigh(637.1)stomatalconductancefollowed by vegetative condition (575.7). At Wudil, the result showed that at 4WAT and 12WAT, there weresignificant ($p<0.05$) differences in which the highest (44.67) stomatal conductance was found in grain filling whilelowest stomatal conductance (5.51) was recorded at vegetative condition in 4WAT.At 12WAT highest stomatalconductance were found at vegetative stage (61.8) with the least (7.5) at grain filling at 2021 season.

Table4: Effect of Fish Guano and Abscisic Acid Concentrations on Stomatal Conductance (mmol m^2s^{-1}) per plant ofgrain amaranth under moisture stress conditions at BUK and Wudil during 2021 and 2022 dry seasons.

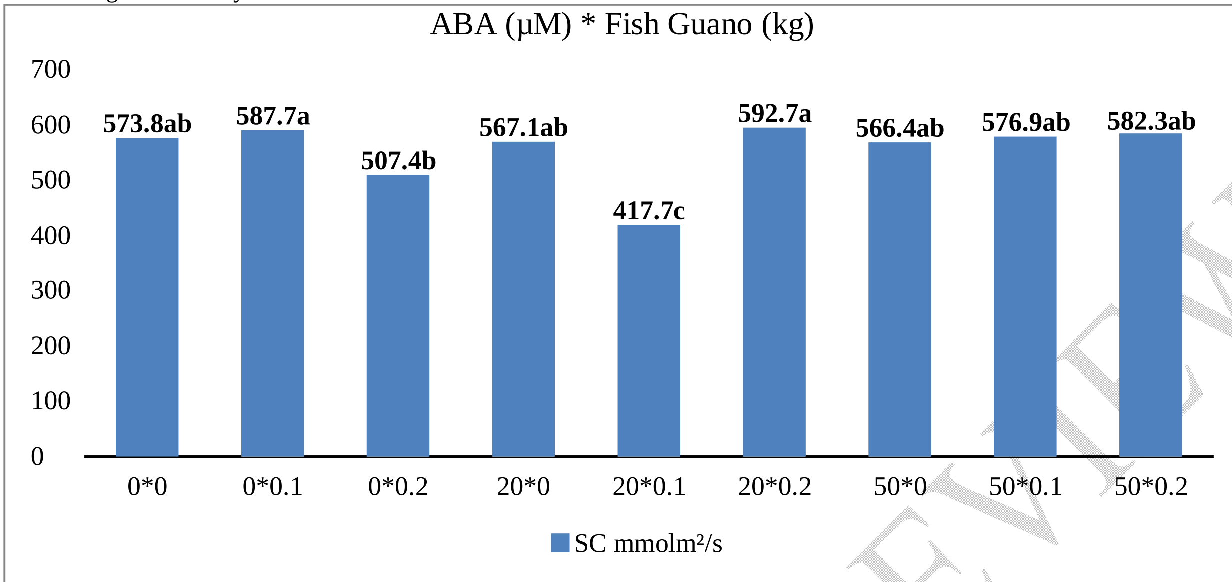
	BUK	Wudil

	2021			2022			2021			2022		
	Weeks after transplanting (WAT)											
Treatments	4	8	12	4	8	12	4	8	12	4	8	12
Stress (S)												
Vegetative	5.83^{b}	37.0	63.8^{a}	74.7	30.47	575.7^{a}	5.51^{b}	37.9	61.8^{a}	10.8	37.7	61.4^{a}
Flowering	46.90^{a}	17.9	50.7^{b}	74.4	30.63	444.9^{b}	42.12^{a}	44.0	49.5^{a}	14.1	44.1	50.0^{a}
Grain Filling	47.64^{a}	51.0	8.1^{c}	74.0	31.08	637.1^{a}	44.67^{a}	23.8	7.5^{b}	6.9	23.8	7.50^{b}
p-value	<.001	0.336	<.001	0.992	0.727	0.008	0.001	0.706	<.001	0.697	0.710	<.001
SE±	2.336	13.81	3.21	3.88	0.537	21.90	2.954	16.74	3.58	5.73	16.74	3.59
Fish Guano (F) (kg)												
0	33.37	33.8	41.9	76.6	30.53	569.1	30.44	35.4	41.0	15.0	35.4	41.1
0.1	33.13	37.0	40.4	74.8	30.36	527.7	32.13	35.5	38.3	9.8	35.5	38.4
0.2	33.88	35.1	40.2	71.6	31.29	560.8	29.74	34.8	39.5	7.1	34.8	39.5
p-value	0.935	0.512	0.812	0.323	0.232	0.144	0.534	0.963	0.658	0.201	0.964	0.660
SE±	1.483	1.94	2.01	2.34	0.405	15.41	1.540	1.99	1.95	3.15	1.98	1.95
ABA (A) (μM)												
0	33.53^{ab}	34.6	41.3	73.7	30.50	556.6	28.79	35.6	40.0	8.9	35.6	40.0
20	36.27^{a}	35.2	41.6	73.5	30.86	525.8	30.41	34.4	40.2	12.0	34.5	40.2
50	30.57^{b}	36.1	39.6	75.9	30.81	575.2	33.11	35.7	38.7	11.0	35.7	38.7
p-value	0.032	0.846	0.759	0.735	0.797	0.083	0.146	0.872	0.831	0.774	0.872	0.831
SE±	1.483	1.94	2.01	2.34	0.405	15.41	1.540	1.98	1.95	3.15	1.98	1.95
Interaction												
S*F	0.686	0.306	0.873	0.055	0.765	0.155	0.218	0.630	0.727	0.786	0.630	0.727
S*A	0.608	0.439	0.933	0.349	0.214	0.091	0.532	0.074	0.754	0.512	0.074	0.754
F*A	0.908	0.929	0.368	0.527	0.157	<.001	0.545	0.737	0.321	0.252	0.737	0.321
S*F*A	0.117	0.861	0.143	0.733	0.313	0.254	0.215	0.564	0.080	0.423	0.564	0.080

Means followed by same letter in a column within a treatment group are not significantly different at 5% level probability using SNK

At Wudil, in 2022, the result revealed that there were no significant (p>0.05) differences on stomatal conductance at4 and 8WAT while significant (p<0.05) difference was recorded at 12WAT in which vegetative state had the highest(61.8) followed by 49.5 while the least was recorded at grain filled condition with 7.50 stomatal conductance in2022. The result on the effect of FG on stomatal conductance of grain amaranth revealed no significant (p>0.05)differences at BUK and Wudil at 2021 and 2022 seasons. In responses to the effect of ABA the result showed thatthere was only significant (p<0.05) differences at 4WAT at BUK in 2021 while others no significant (p>0.05)differences on stomatal conductance were observed in both BUK 2022 and Wudil 2021 and 2022 seasons. Theinteraction result indicates significant (p<0.05) interactions of fish guano and ABA at 12WAT at BUK in 2022season. The effect of fish guano x ABA interaction on stomatal conductance at 12 WAT at BUK during 2022 dryseason is presented in Table 9. The result revealed that there were higher (p<0.05) stomatal conductance interactionbetween 0.2kg FG and 20μM ABA (592.7) followed 0.1kg FG concentration and 0μM ABA (587.7) while loweststomatal conductance interactions were recorded at 0.1kg FG x 20μM ABA concentration level.

Figure 3:Fish Guano x Abscisic Acid interaction on stomatal conductance (mmol $m^2 s^{-1}$) of grain Amaranth at 12WAT at BUKduring the 2022 dry season



Means followed by same letter in a column within a treatment group are not significantly different at 5% level probability using SNK

4 Discussion

Stress had significant effect on the SC of grain amaranth at BUK and Wudil in 2021 and 2022. The significantincreases may be attributed to external influences of environmental factors that are responsible for certain stress likeair temperature rises will increase SC, independent of plant water status and photosynthesis (Jeanguenin*et al*., 2016).Light may be the primary external factor that determines SC as stomata are activated and open during daylight; maybe due to humidity since stomata will open in high humidity even if the leaf water content is less or may be due tosoil water and nutrient status which will also impact stomatal conductance, which will be high when soil moisture ishigh and decreases when soil moisture is less (Buckley, 2019). The PAR was significantly affected by stress at BUKin 2022. Photosynthesis in plants is inhibited due to a loss of balance between the production of reactive oxygenspeciesandantioxidantdefense(FuandHuang2001;Reddy*etal*.,2004).By means of osmotic adjustment, the plantorganellesandcytoplasmic activities take place at about a normal pace and helps plants to perform well in terms of growthand photosynthesis and assimilate partitioning to grain filling (Hussain *et al*.,2016). Thecapacityofplantstoutilizethelightabsorbedbythemdeclinessignificantlywhentheyareexposedtoenvironmental stresses such as drought, salinity, low temperature and high light intensity (Hussain *et al*.,2016). Stress showed a significant effect on the RWC of grain amaranth at BUK. During stomatal closure, decreasedgas exchange results in the reduction of photosynthate production while decreased transpiration can reduce waterloss from leaves (Suzuki *et al*. 2013; Mittler and Blumwald, 2015). In drought conditions, ABA alteration of guardcell ion transport promotes stomatal closure and prevents stomatal opening, reducing water loss (Kim *et al*., 2010). Insunflower water shortage at the flowering stage reduced yield by 29 % (Velue and Palanisami, 2001).

Leaves are the major eco-physiological parts of a plant that interact with the atmosphere (Addai and Alimiyawo,2015)andFGhadasignificanteffectontheLAIofgrainamaranthatWudil2022.Thesignificantdifferences observed from the LAI due to the foliar application of FG tea fertilizer to the leaves were in agreement with theassertions of Davies (2009) who stated that FG is a high-quality organic fertilizer that contains natural ingredientsthatcanpromotevigorousgrowthforoutdoorvegetables,flowers,fruit,trees,ornamentalplants,andindoorhouse plants.ThePARandRWCweresignificantlyaffectedbyFGatBUK.Fishguanodepositionandabsorptionby the leaves may have altered some cellular functions of the plant which causes the stomata to close, as in the waterdeficit case (Lassouane*et al.*, 2013).

The concentration of ABA significantly affects the SC and the RWC of grain amaranth at BUK and the LAI of grainamaranth at both locations. Similar results have been reported by Goreta*et al.* (2007) reported that reductions in SCby ABA enabled the maintenance of leaf water potential and prevented increases in electrolyte leakage and leafabscission. It is well documented that ABA acts as a stress signal, which triggers adaptive changes in the physiologyand morphology of plants (Taiz and Zeiger, 2002). These findings may be linked to the concentration of the ABA inthe xylem sap(CarminatiandJavaux,2020)ormaybefurther attributed to the internal influences (plant-levelfactors) such as signals from the guard cell and stomatal density, changes in leaf water potential, need to take $CO_2$for photosynthesis or association with arbuscularmycorrhizal fungi which form symbiotic associations with 80% ofplant species, can change SC (Kirkham, 2014).

In drought conditions, ABA alteration of guard cell ion transport promotes stomatal closure and prevents stomatalopening, reducing water loss (Kim *et al.*, 2010). Muhammad *et al.* (2022)found that bell pepper seedlings dipped inNiABAsolutionhadhigherstomatalresistanceandleafwaterpotentialthanuntreatedseedlingsafter transplanting. It is also known that ABA can limit transpirational water loss by inducing stomatal closureand inhibition of leaf growth or expansion(Shinsuke and Daniel,2012).Several studies reported that restricted leaf expansion was correlated with ABA increases in xylem sap (Ismail *et al.*,2002) or leaves (Alves and Setter 2000). A reduction in the leaf area of sunflowers has been observed under ac continuous 30-day drought period, ending at anthesis rather than full irrigation (Hammadeh*et al.*,2005). In recentresults on spinach production, a foliar application using S-ABA ConTego effectively held leaf size at its marketablevalue even before the harvesting period (Racsko*et al.*, 2014).

The exogenous application of ABA affected water use efficiency more in the droughty plants than well-wateredplants of jewelweed (Heschel and Hausmann, 2001). Similarfindings have been reported by Goreta*et al.* (2007). Therelative water content of grain amaranth obtained in this study was similar to those reported by Cechi*et al.* (2022) that foliar sprays of ABA applied before withholding water to muskmelon seedlings improved maintenance of leaf water potential and RWC thus minimizing dehydration-induced damage to membranes.

5 Conclusion

The use of Abscisic acid at concentrations of 50µM and 0.2 kg Fish Guano plays a vital role in regulating plantgrowth and development, especially in conditions of water scarcity. The combined application of fish guano andABA demonstrated noteworthy effects on water relations grain amaranth plant exposed to moisture stress conditions atdifferentstages,includingthevegetative,flowering,andgrain-fillingphases.Thesefindings highlighted thepotential of fish guano and ABA as valuable tools for enhancing the resilience and performance of grain amaranth crops inwater-limited environments.The use of Abscisic acid at concentrations of 50 µM and FG at 0.2 kg plays a vital role in regulating plant growth and development, especially in moisture stress condition. The combined application of FG and ABA demonstrated remarkable effects on water relations of grain amaranth plant exposed to moisture stress conditions at different growth stages, including the vegetative, flowering, and grain-filling phases. These findings highlighted the potentialof FG and ABA as valuable tools for enhancing the resilience and performance of grain amaranth crops in drought prone environments.

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1.

2.

3.

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