

ORIGINAL RESEARCH ARTICLE

Screening of *Vigna subterranean* L. Verdc. accessions for waterlogging stress tolerance

Okon Godwin Okon^{1,*}, Augustine Effiong Archibong¹, Ofonime Raphael Akata², Imikan Anyieokpon Nyong¹, Ekomobong Etinam Akpan¹

¹ Department of Botany, Akwa Ibom State University, UYO P.M.B. 1167, Nigeria

² Department of Crop Science, Akwa Ibom State University, UYO P.M.B. 1167, Nigeria

*Corresponding author: Okon Godwin Okon, okonokon@aksu.edu.ng

ABSTRACT

This study evaluated the influence of waterlogging stress on the growth of six (6) accessions (TvSu-1, TvSu-2, TvSu-3, TvSu-4, TvSu-5, and TvSu-10) of *Vigna subterranean* in a pot experiment. The experiment was setup in a complete block design (CBD) with 3 replicates per treatment. Results of growth parameters of *V. subterranean* accessions under waterlogging stress such as; plant height, leaf area, petiole length, and number of nodes, were significantly ($p = 0.05$) decreased when compared to their controls after 8 weeks of planting. For shoot length, TvSu-2 (1.60 ± 0.20 cm) and TvSu-4 (1.60 ± 0.20 cm) recorded the highest values while TvSu-5 (1.23 ± 0.03) and TvSu-10 (1.23 ± 0.03) had the lowest values respectively. TvSu-5 (19.03 ± 0.59 cm²) and TvSu-10 (19.03 ± 0.59 cm²) recorded the highest values in leaf area (LA), while TvSu-3 (14.40 ± 0.51 cm²) had the lowest LA. For total photosynthetic pigment (TPP); TvSu-2, TvSu-4, TvSu-10 had the highest values with 57.35 ± 1.82 mg/kg, 55.80 ± 2.70 mg/kg, 55.77 ± 1.90 mg/kg respectively, TvSu-3 (41.50 ± 8.29 mg/kg) maintained the lowest value. In petiole length, TvSu-5 (15.23 ± 0.33 cm) and TvSu-4 (14.20 ± 0.66 cm) had the highest values, while TvSu-3 (8.97 ± 0.33 cm) had the lowest. For number of nodes, TvSu-2 (15.00 ± 1.76) and TvSu-4 (12.00 ± 1.73) recorded the highest values, while TvSu-10 (10.00 ± 1.00) had the lowest value. Biomass yield analysis of the stressed *V. subterranean* showed that total fresh weight (TFW), root length (RL), root fresh weight (RFW), shoot fresh weight (SFW), leaf fresh weight (LFW), leaf turgid weight (LTW), total dry weight (TDW), root dry weight (RDW), shoot dry weight (SDW) and leaf dry weight (LDW) of the six accessions were significantly ($p = 0.05$) decreased when compared to their control. TvSu-5 had a better biomass yield when compared to other accessions recording the highest values in SDW (1.62 g), RDW (0.55 g), LFW (0.75 g), SFW (10.31 g) and RL (18.53 ± 0.66). Conclusively, waterlogging stress negatively impacted *V. subterranean* accessions, but TvSu-5 had a better waterlogging stress tolerance than other accessions especially TvSu-3, which was generally poor.

Keywords: Bambara groundnut; cowpea; stress tolerance; *Vigna subterranean*; waterlogging

1. Introduction

Native to Africa, the Bambara groundnut (*Vigna subterranean* L. Verdc) is a leguminous crop cultivated mostly for its seeds that is becoming more and more well-liked as a food source in rural regions all throughout the continent. After cowpea (*Vigna unguiculata*) and peanut (*Arachis hypogea*), it is the third most significant legume^[1]. In developing countries, it is crucial to improve the consumption of food legumes

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like bambara groundnuts since they have significant potential to combat malnutrition^[2-4]. Bambara nuts are considered a complete diet due to their high protein level (18%) and high carbohydrate content (65%). It is cultivated mostly for its highly nutritious seeds^[5] which humans consume^[6]. According to Adeleke et al.^[6], the seeds are used in many different types of cuisine, some of which are essential to human diets.

However, Bambara groundnut is still grown from local landraces rather than from kinds selected specifically for certain locations, and farm yields are still poor despite its importance in the subsistence diet of much of Africa. Many people believe that Bambara groundnuts can withstand droughts^[7]. On the other hand, water stress reduces Bambara's area of the leaf (LAI) and seed germination^[8]. Grown extensively in tropical and sub-tropical countries, Bambara groundnut (*Vigna subterranean*) is regarded as a crop vulnerable to soil waterlogging^[9-11]. Two of the earliest indications of water stress in plants are decreased leaf development and stomata closure^[12]. Numerous legumes have been shown to have significant yield reductions after just one day of water logging^[13,14].

A significant barrier to the production of many crops is waterlogging^[15]. Prolonged seasonal rainfall is the primary cause of severe crop losses in tropical and subtropical regions. Although many plant species can develop a combination of mechanisms enabling them to grow under flooding, waterlogging significantly reduces the productivity of plants^[16]. When plants are waterlogged, their primary restriction is an oxygen shortage^[17,18].

The main variables influencing plant performance during waterlogging are the length and depth of soil submersion as well as the stage of growth of the plants. It is generally accepted that the negative effects on plants increase with the length of the waterlogging period. The rate of water input, the amount of water flowing out of the rooting zone, and the soil's ability to absorb water all affect how severely waterlogging damages plants. However, oxygen must be added to the interior of roots in amounts that support respiration and processes like mineral and water uptake if the plant is to survive longer periods of time in oxygen-deficient environments^[19]. According to Greenway et al.^[20], the crops, growth habits, and length of waterlogging could all affect the survival mechanism. The physiological, morphological, and molecular aspects of Bambara groundnut are impacted by water scarcity^[21]. Water stress causes Bambara plants' photosynthesis and chlorophyll content to drop, which slows the plant's growth and prematurely ages it^[22]. According to a few studies, Bambara production and the total number of seeds are decreased by water stress. According to Mabhaudhi et al.^[12], Bambara pod production is decreased by water stress. Plant growth and productivity are impacted by water stress^[23].

The frequency, length, and timing of the waterlogging event(s) in relation to the crop's growth stage determine how much crop yield is affected^[24]. According to Cannell et al.^[25], waterlogging during germination, or the early stages of vegetative growth, has the biggest negative impact on crop yields. The goal of this research is to evaluate how the total photosynthetic pigments, biomass yield, and growth of Bambara groundnut are affected by waterlogging.

2. Materials and methods

2.1. Study area

The experiment carried out in Akwa Ibom State University, (AKSU), Ikot Akpaden, Mkpato Enin. Mkpato Enin is located in the south-south region of Nigeria and it is a local government area in Akwa Ibom State. Its altitude is about 185 m. The local government has an area of 322,352 km and it is located within the industrial belt extending from Eastern Obolo, Etinan, Oruk anam, Onna to Ikot Abasi. The experiment was carried out between February to April 2023.

2.2. Experimental materials and sources

Six accessions of *Vigna subterranea* TVSu-1, TVSu-2, TVSu-3, TVSu-4, TVSu-5 and TVSu-10 were collected from the International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. Seed coat colour and size were physically observed and documented. The soil used in planting was gotten from Akwa Ibom State University Campus compound. The experiment was setup in a randomized complete block design (4 treatment, 3 replicate which each treatment has its control) in at the Department of Botany, Akwa Ibom State University, Ikot Akpaden, Akwa Ibom State.

2.3. Planting

Five seeds were sown at 3 cm depth in each planting bucket (10 L) filled with loamy soil 3 replicates were made for each accessions, well labelled and laid out in a randomized complete block design (RCBD). Water logging stress was done by adding water to the unperforated bucket at 2 cm above soil level. No fertilizer or pesticides was applied, soil was sterilized to kill all weed seeds.

2.4. Determination of shoot length (cm)

The shoot length was obtained by measuring the heights of the plant using a ruler.

2.5. Determination of leaf area (cm²)

The leaf area (LA) was determined every two weeks after sprouting. Measurements were taken using measuring tape; the area (A) of the leaf was determined by tracing the outline of the activities on the leaf. The area covered by the outline was then calculated. The correlation factor (K) was determined by dividing the area (A) by product of length x breadth of the leaf. Therefore, the leaf area for each plant was determined using the formula:

$$A = L \times B \times K$$

where A = Leaf length, L = Leaf length, B = Leaf width, K = Correlation factor. The correlation factor (K) for *Vigna subterranea* L. Verdc was 0.75.

2.6. Determination of petiole length (cm)

The petiole length was determined by measuring the length or heights of the petiole in plant using a ruler.

2.7. Determination of total photosynthetic pigment

Total photosynthetic pigments content of the test plant was determined using atLeaf chlorophyll meter, PN: 0131FT: GREEN LLC. USA.SN: 0903-0100145. The leaf was inserted into the sensor to determine its chlorophyll content value which was displayed on the chlorophyll meter screen.

2.8. Determination of number of nodes

The number of nodes was determined by carefully counting the number of leaves that had developed on each plant.

2.9. Statistical analysis

All data in the present study were subjected to analysis of variance (ANOVA) using GraphPad Prism and data are presented as standard error of mean (\pm S.E.M.) of triplicate experiments. The differences between the means were separated and compared using the Duncan's multiple range tests. However, a probability level of $p = 0.05$ was considered statistically significant.

3. Results and discussion

The result of growth analysis of growth parameters of bambara groundnut such as plant height, leaf area, petiole length, number of nodes and photosynthetic pigment were significantly ($p = 0.05$) decreased when compared to the control at 2 weeks of growth (**Table 1**).

At 4 weeks, TvSu-1 and TvSu-3 recorded the highest value for waterlogging treated plants in plant height with 1.17 ± 0.27 cm and 1.17 ± 0.27 cm respectively. For leaf area, TvSu-5 and TvSu-10 recorded the highest value with 15.90 ± 1.15 cm² and 15.90 ± 1.15 cm² respectively. TvSu-1 had the highest photosynthetic pigment with the value 54.27 ± 2.09 mg/kg followed by TvSu-2 (52.83 ± 0.49 mg/kg), TvSu-4 (52.20 ± 1.75 mg/kg), TvSu-3 (51.90 ± 3.70 mg/kg), TvSu-5 (48.80 ± 1.79 mg/kg). TvSu-10 had the least photosynthetic pigment with the value 46.50 ± 3.16 mg/kg. TvSu-5 recorded the highest value 14.83 ± 0.32 cm for petiole length, followed by TvSu-4 (14.03 ± 0.68 cm), TvSu-1 (12.10 ± 0.61 cm), TvSu-10 (11.43 ± 1.45 cm), TvSu-2 (10.66 ± 1.30 cm), while TvSu-3 had the least value with 8.57 ± 0.27 cm. TvSu-5 and TvSu-3 had the highest number of nodes with the values 11 ± 1.73 and 11 ± 1.00 respectively. TvSu-5 showed better tolerance to waterlogging than other accessions, having recorded the highest values for leaf area, petiole length and number of nodes with average height, followed by TvSu-3.

Table 1. The effects of waterlogging on some growth parameters of *Vigna subterranea* at 4 weeks after planting (WAP).

Treatment		Plant height (cm)	Leaf area (cm ²)	Photosynthetic pigment (mg/kg)	Petiole length (cm)	Number of nodes
TvSu-1	T	1.17 ± 0.27^a	12.30 ± 1.30^a	54.27 ± 2.09^a	12.10 ± 0.61^a	9.00 ± 1.00^a
	C	1.10 ± 1.00^a	15.00 ± 0.69^b	50.63 ± 1.05^b	11.76 ± 1.01^a	12.00 ± 1.00^b
TvSu-2	T	1.00 ± 0.100^a	12.70 ± 1.54^a	52.83 ± 0.49^a	10.66 ± 1.30^a	9.00 ± 1.00^a
	C	1.00 ± 1.00^a	16.40 ± 0.71^b	52.33 ± 4.18^a	12.33 ± 0.91^a	11.00 ± 1.90^b
TvSu-3	T	1.17 ± 0.27^a	11.30 ± 1.60^a	51.90 ± 3.70^b	8.57 ± 0.27^b	11.00 ± 1.00^b
	C	1.10 ± 1.00^a	12.00 ± 0.69^a	50.60 ± 1.48^b	10.53 ± 0.75^a	14.00 ± 1.00^c
TvSu-4	T	1.00 ± 0.100^a	12.70 ± 1.54^a	52.20 ± 1.75^a	14.03 ± 0.68^c	10.00 ± 1.00^b
	C	1.00 ± 1.00^a	16.40 ± 0.71^b	54.50 ± 0.51^a	13.37 ± 0.64^c	12.00 ± 1.73^b
TvSu-5	T	1.00 ± 1.00^a	15.90 ± 1.15^b	48.80 ± 1.79^c	14.83 ± 0.32^c	11.00 ± 1.73^b
	C	1.60 ± 0.00^b	13.80 ± 1.95^b	46.50 ± 1.89^d	15.20 ± 0.40^c	12.00 ± 0.00^b
TvSu-10	T	1.00 ± 1.00^a	15.90 ± 1.15^b	46.77 ± 1.31^d	11.43 ± 1.45^a	10.00 ± 1.78^b
	C	1.60 ± 0.00^b	13.80 ± 1.95^b	46.50 ± 3.16^d	10.27 ± 0.46^a	13.00 ± 0.00^b

T: Waterlogging treatment, C: Control. Mean^{abc} data with same letters are not statistically significant.

At 8 weeks after planting, TvSu-2 and TvSu-4 recorded the highest value in growth for waterlogging treated plants in plant height with 1.60 ± 0.20 cm and 1.60 ± 0.20 cm respectively. For leaf area, TvSu-5 and TvSu-10 recorded the highest value with 19.03 ± 0.59 cm² and 19.03 ± 0.59 cm² respectively. TvSu-2 had the highest total photosynthetic pigment with the value 57.35 ± 1.82 mg/kg followed by TvSu-4 (55.80 ± 2.71 mg/kg) and TvSu-10 (55.77 ± 1.90 mg/kg), TvSu-1 (54.30 ± 2.18 mg/kg), TvSu-5 (50.90 ± 2.07 mg/kg). TvSu-3 had the least total photosynthetic pigment with the value 41.80 ± 8.29 mg/kg. TvSu-5 recorded the highest value 15.23 ± 0.33 cm for petiole length, followed by TvSu-4 (14.20 ± 0.66 cm), TvSu-10 (10.87 ± 1.22 cm), TvSu-2 (10.85 ± 0.82 cm), TvSu-1 (10.25 ± 0.25 cm), while TvSu-3 had the least value with 8.97 ± 0.23 cm. TvSu-2 had the highest number of nodes (15.00 ± 1.76), followed by TvSu-4 and TvSu-3 (12.00 ± 1.73 and 12 ± 0.00 respectively), TvSu-5 (11.00 ± 1.00), while TvSu-1 and TvSu-10 recorded the least number of nodes (10 ± 0.33 and 10.00 ± 1.00 , respectively). TvSu-2 followed by TvSu-4 showed better

tolerance to waterlogging stress at 8 weeks. TvSu-2 recorded the highest value for plant height, photosynthetic pigment, number of nodes and average leaf area (**Table 2**).

Table 2. The effects of water logging on some growth parameters of *Vigna subterranean* at 8 weeks after planting.

Treatment		Plant height (cm)	Leaf area (cm ²)	Photosynthetic pigment (mg/kg)	Petiole length (cm)	Number of nodes
TvSu-1	T	1.33 ± 0.26 ^a	16.40 ± 0.51 ^a	54.30 ± 2.18 ^a	10.25 ± 0.25 ^a	10 ± 0.33 ^a
	C	1.37 ± 0.03 ^a	17.55 ± 0.69 ^a	49.62 ± 0.87 ^b	11.10 ± 1.01 ^a	16 ± 3.28 ^b
TvSu-2	T	1.60 ± 0.20 ^b	16.67 ± 0.48 ^a	57.35 ± 1.82 ^c	10.85 ± 0.82 ^a	15 ± 1.76 ^b
	C	1.80 ± 0.00 ^c	28.07 ± 2.19 ^b	57.60 ± 2.09 ^c	10.92 ± 0.95 ^a	15 ± 1.53 ^b
TvSu-3	T	1.33 ± 0.26 ^a	14.40 ± 0.51 ^a	41.80 ± 8.29 ^d	8.97 ± 0.23 ^b	12 ± 0.00 ^a
	C	1.37 ± 0.03 ^a	14.57 ± 0.69 ^a	51.90 ± 3.44 ^a	10.97 ± 0.43 ^a	21 ± 3.00 ^c
TvSu-4	T	1.60 ± 0.20 ^b	15.67 ± 0.48 ^a	55.80 ± 2.71 ^a	14.20 ± 0.66 ^c	12 ± 1.73 ^a
	C	1.80 ± 0.00 ^c	28.07 ± 2.19 ^b	54.70 ± 0.82 ^a	15.43 ± 1.14 ^c	13 ± 1.53 ^a
TvSu-5	T	1.23 ± 0.03 ^d	19.03 ± 0.59 ^c	51.80 ± 1.96 ^a	15.23 ± 0.33 ^c	11 ± 1.00 ^a
	C	1.80 ± 0.00 ^c	23.63 ± 1.52 ^c	50.90 ± 2.07 ^a	16.07 ± 0.60 ^c	15 ± 0.00 ^b
TvSu-10	T	1.23 ± 0.03 ^d	19.03 ± 0.59 ^d	55.77 ± 1.90 ^a	10.87 ± 1.22 ^a	10 ± 1.00 ^a
	C	1.80 ± 0.00 ^c	23.63 ± 1.52 ^c	53.81 ± 2.41 ^a	11.55 ± 1.22 ^a	18 ± 0.00 ^d

T: Waterlogging treatment, C: Control. Mean^{abc} data with same letters are not statistically significant.

After 8 weeks of planting, the biomass yields of the six accessions of *Vigna subterranean* were analyzed. TvSu-1 and TvSu-10 had the highest total fresh weight 3.68 ± 0.01 g and 3.67 ± 0.00 g respectively. TvSu-2, TvSu-5 and TvSu-10 recorded the highest value for root length 18.60 ± 1.40 cm, 18.53 ± 0.66 cm and 18.00 ± 0.45 cm respectively, followed by TvSu-4 (16.40 ± 1.43 cm). TvSu-4 had the highest value (5.25 g) for root fresh weight, followed by TvSu-3 and TvSu-5 (4.99 g and 4.27 g) respectively. TvSu-5 recorded the highest value (10.31 g) for shoot fresh weight, TvSu-4 had 9.07 g, TvSu-2 had the least value (3.07 g). For Leaf fresh weight, TvSu-5 recorded the highest value (0.75 g) while TvSu-2 had the least value (0.20 g). TvSu-2 and TvSu-10 had the highest value (1.40 g and 1.40 g) respectively, for leaf turgid weight, followed by TvSu-5 (1.28 g). For total dry weight TvSu-1 and TvSu-3 recorded the highest value (1.58 g and 1.58 g). TvSu-5 and TvSu-10 recorded the highest value for root dry weight (0.55 g and 0.55 g) respectively, followed by TvSu-2 and TvSu-4 (0.41 g and 0.41 g). TvSu-5 and TvSu-10 also recorded the highest value for shoot dry weight (1.62 g and 1.62 g) respectively. For leaf dry weight, TvSu-4 had the highest value 0.71 g, followed by TvSu-2 (0.67 g), while TvSu-1 had the least value (0.24 g). Among the six (6) accessions of *Vigna subterranean*, TvSu-10 and TvSu-5 had the highest biomass yield. TvSu-10 and TvSu-5 had the highest biomass yield for root length, root dry weight and shoot dry weight. TvSu-10 recorded the highest biomass for total fresh weight and leaf turgid weight, while TvSu-5 recorded the highest biomass yield for shoot fresh weight and leaf fresh weight (**Table 3**).

Table 3. Biomass yield of *Vigna subterranea* under waterlogging stress.

Treatment		Total fresh weight (g)	Root length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Leaf fresh weight (g)	Leaf turgid weight (g)	Total dry weight (g)	Root dry weight (g)	Shoot dry weight (g)	Leaf dry weight (g)
TvSu-1	T	3.68 ± 0.01 ^a	14.00 ± 1.60 ^a	0.40	3.29	0.28	0.85	1.58	0.40	1.24	0.24
	C	3.53 ± 0.00 ^b	22.00 ± 2.22 ^b	2.70	10.02	0.29	0.80	0.53	0.48	1.64	0.22
TvSu-2	T	3.61 ± 0.00 ^a	18.60 ± 1.40 ^c	1.53	3.07	0.20	1.40	1.06	0.41	1.39	0.67
	C	3.73 ± 0.00 ^c	22.00 ± 1.60 ^b	2.61	2.76	0.24	0.90	1.26	0.50	0.59	0.13
TvSu-3	T	3.61 ± 0.01 ^a	15.07 ± 1.79 ^a	4.99	6.62	0.54	0.97	1.58	0.40	1.24	0.26
	C	3.59 ± 0.00 ^a	20.17 ± 2.11 ^b	3.45	7.01	0.43	0.91	0.53	0.48	1.64	0.24
TvSu-4	T	3.57 ± 0.00 ^c	16.40 ± 1.43 ^a	5.25	9.07	0.72	1.09	1.06	0.41	1.39	0.71
	C	3.56 ± 0.00 ^c	18.70 ± 1.79 ^c	5.02	15.31	0.78	0.95	1.26	0.50	0.59	0.13
TvSu-5	T	3.54 ± 0.00 ^c	18.53 ± 0.66 ^c	4.27	10.31	0.75	1.28	0.21	0.55	1.62	0.38
	C	3.54 ± 0.00 ^c	20.17 ± 1.88 ^b	3.87	13.47	0.58	0.98	1.86	0.64	1.50	0.21
TvSu-10	T	3.67 ± 0.00 ^a	18.00 ± 0.45 ^c	1.84	5.62	0.32	1.40	0.21	0.55	1.62	0.29
	C	3.70 ± 0.00 ^c	20.50 ± 1.95 ^b	2.07	6.18	0.19	0.80	1.86	0.64	1.50	0.19

TFW = Total fresh weight, RL = Root length, RFW = Root fresh weight, SFW = Shoot fresh weight, LFW = Leaf fresh weight, LTW = Leaf turgid weight, TDWR = Total dry weight root/shoot, TRDW = Root dry weight, SDW = Shoot dry weight, LDW = Leaf dry weight. Mean^{abc} data with same letters are not statistically significant.

The six (6) *Vigna subterranea* accessions displayed varying degrees of waterlogging tolerance, some accessions fared better under waterlogging stress condition than others. This plant's ability to withstand waterlogging resulted from the development of a fibrous root system outside the soil at the base of the stem, which aided the plant in absorbing water and nutrients and exchanging gases that are essential for plant growth. The findings of this investigation are consistent with those of Vassar and Voeselek^[26], who observed that the development of the fibrous roots system (FRS) is a common adaptive alteration in morphology. TRS form in the internodes at the base of the stem or on the hypocotyl during prolonged waterlogging, where they facilitate the exchange of gases is the uptake of nutrients and water. The primary roots that perish from hypoxia stress can be partially replaced by FR formation, which preserves metabolic cycles and permits normal growth and development. Compared to the primary roots, the newly formed FRS have more aerenchyma, which improves O₂ uptake and diffusion capacity. Aerenchyma can release toxic volatile substances and CO₂ from wet tissue in addition to transferring oxygen from dry tissue to the root system. The results agrees with the study also agrees to the report by Mabhaudhi et al.^[8], that the growth parameters of bambara were reduced by water stress.

The findings for biomass yield revealed that the six (6) accessions' root length, leaf turgid weight, root dry weight, and leaf dry weight were all lower than those of the control. Nonetheless, Tvsu-1 and Tvsu-3's total fresh weight was greater than the control's. Likewise, Tvsu-3, Tvsu-4, and Tvsu-5 had greater root fresh weights than the control. With the exception of Tvsu-2, the shoot fresh weight of accessions was decreased. Tvsu-1 and Tvsu-3 had greater total dry weights than the control, as did Tvsu-3, Tvsu-5, and Tvsu-10 leaf fresh weights. Additionally, compared to the control, the shoot dry weight for Tvsu-2, Tvsu-4, Tvsu-5, and Tvsu-10 was higher.

Based on observations, the growth parameters and biomass yield evaluated, Tvsu-5 shows a higher tolerance for waterlogging. Waterlogging had a detrimental effect on the growth of *Vigna subterranea* because, at week 8, all accessions' growth parameters were significantly reduced under waterlogging stress, especially when compared to their controls (**Figure 1**).



Figure 1. Effects of waterlogging on some accessions *Vigna subterranean*.

4. Conclusion

This study assessed the effects of waterlogging stress on six Bambara groundnut accessions' growth parameters. The plants' ability to grow was significantly impacted by waterlogging stress. Tvsu-5 showed the highest tolerance to waterlogging among the six accessions based on biomass yield and growth parameters as a result of their physical characteristics in relation to other accessions but when compared to their controls. Thus, waterlogging stress negatively impacted the Bambara groundnut's growth parameters. Tvsu-5 is better suited to waterlogged areas.

Author contributions

Conceptualization, OGO; methodology, AEA; investigation, ORA; resources, OGO; data curation, EEA; writing—original draft preparation, AEA; writing—review and editing, IAN; project administration, OGO; funding acquisition, OGO. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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