Effect of phosphorus fertilizer on morphological, physiological, and biochemical traits of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) plants underneath water deficit

ABSTRACT

Aims: To evaluate the effect of water deficit (WD) and P-fertilizer on the growth and biochemical composition of Bambara groundnut.
Study design: The experimental design was a randomized complete block with three factors: landraces (L1, L2, and L3), single superphosphate doses (0, 20, 40, 60, 100 mg $P_2O_5.kg^{-1}$), and watering regime (90% (control), 60%, and 30% of field capacity (FC)).
Place and Duration of Study: The experiment was conducted in the shelter at the University of Yaounde I for three months.
Methodology: The seedlings (radicle at 2 mm) were sown in the polyethylene pots filled with substrate with the appropriate P-doses. The plants were grown for up to four weeks under normal watering level (90%_FC). Four weeks after sowing (WAS), the WD was applied by stopping irrigation and maintaining the desired FC. At harvest (8WAS), growth, physiological and biochemical parameters were evaluated.
Results: P-fertilizer significantly improved growth and biochemical composition under WD or none. The plant height increased from 20.3% from 0 to 100 mg P_2O_5 at 30%_FC to L2 at the 8^{th} week. At 90%_FC, the doses 20, 40, 60, 100 mg increased sugars content by 52.6, 59.4, 64.6, and 90.4%, respectively, compared to 0 mg at L3. Proline content at 30%_FC was twice (2.5) that of 90%_FC at L2. Increased accumulation of sugars, proline, and amino acids in leaves was recorded at the severe level of WD.
Conclusion: P-fertilizer mitigated the adverse effect of WD on the growth and biochemical composition of Bambara groundnut. A sufficient P-supply (60 mg $P_2O_5.kg^{-1}$) was recommended to help Bambara groundnut plants tolerate WD. L1 and L3 appear to be more tolerant than L2 and can be recommended to farmers. As the adaptation mechanisms of these landraces to WD are different, it would be advisable to evaluate their performance in the field to better assess their behavior.

Keywords: Bambara-bean; biomass; drought stress; growth; P-fertilizer; proline; sugars.

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1. INTRODUCTION

Pulses are used throughout the world as an essential component in staple foods to fight famine. They are used in cropping systems to fix atmospheric nitrogen and enrich the soil with nitrogen through their symbiosis with rhizobia. The rhizobia-Bambara groundnut symbiosis can fix 32-81 kg N ha^{-1} [1]. *Vigna subterranea* (L.) Verdc, commonly known as Voandzou or Bambara groundnut (BG), is a pulse crop originating from northeastern Nigeria and north of Cameroon [2-4]. It is composed of carbohydrates, minerals (Ca, K, Fe, N, and Mg), vitamins, proteins, and amino acids; it is highly caloric [5-8]. With an average production of 40,640 t in 2022, Cameroon was the third-largest producer after Burkina Faso

and Niger [9]. In Africa in particular, legume cultivation is an integral part of the industrial and agricultural systems of the tropics. On the nutritional level, their energy and protein intake are of great interest. In tropical Africa and Cameroon in particular, the cultivation of BG is experiencing significant falls in yield, often attributed to biotic (pathogenic aggression, disease, competition, etc.) and abiotic constraints (salinity, nutrient deficiency, water deficit, etc.) [10-14]. Indeed, since the 1980s, there has been a decline in the production of legumes in Cameroon, despite the growing demand on the market [7, 15]. Environmental stresses, particularly water and nutrient deficits (especially phosphate deficiency), are also among the main causes of this drastic drop in yields and are important limiting factors for crop development, yield, and survival [12, 16-18]. The problem of water availability is acute with the scarcity of rainfall. In sub-Saharan Africa, drought is common and has a devastating effect on the crops and economies of many countries. The main causes of these droughts are irregularity and/or low rainfall, particularly in the arid and semi-arid areas of the continent. In addition, P is also a limiting element for crop return on more than 30% of the world's cultivated soil [19]. It is the most limiting factor for plant nutrition because of its low availability in the land [16-17, 20]. Several factors influence the mobility of different P-sources. Some studies have shown that increasing P on the soil will increase the plants tolerance to water stress [21]. Could P-fertilization help reduce the harmful effect caused by water deficit on growth of BG? **(References, if any may be added)** Which landrace was better under water deficit condition? This work aims to assess the effect of water deficit and P-fertilization on the growth and biochemical composition of BG.

2. EXPERIMENTAL DETAILS

2.1 Biological material

The biological material consists of three BG (Fig. 1) landraces (ivory cream (L1), brownish red (L2), and ivory cream with grey eyes (L3) seed coats) purchased from the seed markets in Cameroon. They were the most appreciated by the consumers and producers [4, 7].

2.2 Study site

The research was carried out in a shelter (12 hours photoperiod, 25±5°C, 70-80% of moisture, 2400-3500 lux) built at the Faculty of Science of the University of Yaounde I (Cameroon).

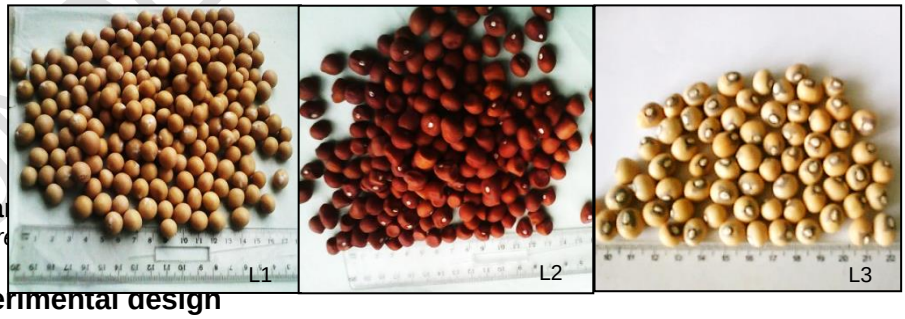

Fig. 1. Ba
L1: ivory cr

2.3 Experimental design

The experimental design was a randomized complete block with three treatments: five P-doses (0, 20, 40, 60, and 100 mg of $P_2O_5.kg^{-1}$ of substrate) [21], three watering levels (control: 90% of field capacity (FC), moderate deficit: 60%_FC, and pronounced deficit: 30%_FC) and three landraces (L1, L2 and L3).

2.4 Determination of field capacity

The physicochemical analysis (Table 1) of the culture substrate (¾ sieved soils + ¼ river sand (2 mm)) was done at the IITA soil's laboratory of Nkolbisson before the trial was set up. The substrate's FC was evaluated [22] as following. The FC is the maximum amount of water a physiologically normal soil can hold for crops. To obtain it, dry substrate was poured into a pot perforated underneath. The weight P1 of the full pot of dry substrate was determined. The substrate was watered to saturation and left to dry for 48 h in the dark. The pot was weighed again to obtain the weight P2. The difference P2-P1 gave the amount of water corresponding to the FC [22].

Table 1. Physical and chemical characteristics of substrate

Sand	Silt	Clay	pH	C	N	C/N	P	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	CEC
	%		-	%		-	$\mu g.g^{-1}$			$cmol.kg^{-1}$		
73.47	7.42	19.11	7.04	2.48	0.13	18.83	9.74	9.69	0.72	0.37	0.06	6.63

C/N: carbon/nitrogen, CEC: cation exchange capacity

2.5 Setting up the experiment

The polyethylene pots (4 L) filled with 3.5 kg of substrate were organized in a randomized complete block design with three repetitions for each treatment. The seedlings (radicle at 2 mm) were then sown with the appropriate P-doses in pots at the proportion of three seeds/pot. The plants were grown for up to four weeks under normal watering level (90%_FC). Four weeks after sowing (WAS), the water deficit was applied by stopping irrigation and maintaining the desired FC. At harvest (eight weeks after sowing, before flowering), growth, physiological and biochemical parameters were evaluated.

2.6 Evaluation of parameters

2.6.1 Morphological (growth) parameters

2.6.1.1 N° of leaves

The N° of leaves was measured every week as soon as the stress was applied (four weeks after sowing (WAS)) until the harvest (8WAS). The N° of leaves was found through counting the N° of new leaves formed. The last formed leaf was marked with a sign at the petiole to facilitate the detection of the new leaves formed each day.

2.6.1.2 Shoot height

The shoot height (cm) was measured every week as soon as the stress was applied (4WAS) until the harvest (8WAS). Shoot height was determined employing a measurement tape.

2.6.1.3 Biomass

Eight weeks after sowing (WAS), the plant samples (whole plants leaf, stem, and root) were harvested and rapidly weighed to obtain their fresh weight. The dry matter weight was obtained by drying the plant material (whole plant, leaf, stem, and root) in an oven at 80°C (72 h). The samples were weighed at regular intervals, until a constant weight was obtained.

2.6.2 Physiological parameters

2.6.2.1 Plant water content

The plant water content (PWC) was obtained by the ensuing principle:

$$PWC = (PFW-PDW) / PDW \quad (1)$$

where PFW represents the fresh weight and PDW the dry weight of the plant.

2.6.2.2 Relative water content

The turgor status was estimated by calculating the relative water content (RWC). Leaves were censored at the base of the blade and directly weighed to get the fresh weight (FW). These leaves were subsequently put in pipes containing distilled water and positioned in the black. After 24 h, the leaves were taken away, passed through absorbent paper to engross water from the surface, and then weighed once more to get the weight at full turgor (FWT). Finally, the samples were put in an oven at 80 °C for 48 h and weighed to get their dry weight (DW). RWC was obtained by the ensuing principle [23]:

$$RWC (\%) = [(FW-DW) / (FWT-DW)] \times 100 \quad (2)$$

Where, RWC: relative water content, FW: fresh leaf weight, DW: dry leaf weight, and FWT: leaf weight at full turgor.

2.6.2.3 Stress Resistance Index

The stress resistance index of plant was obtained based on the dry biomass produced for each level of water stress (30 and 60% FC). It was obtained by the formula described by Fischer and Maurer [24]:

Water stress resistance index (biomass) = (Total dry biomass of stressed plant) / (Total dry biomass of unstressed plant) (3)

2.6.3 Biochemical parameters

2.6.3.1 Extraction

Biochemical parameters (total soluble sugars, proline, and total amino acids) were settled as of an ethanolic extract. One gram of leaf was ground in 10 mL of 80° ethanol and centrifuged at 5,000 rpm at 4°C for 15 min by a centrifuge (JOAN). The recovered supernatant was utilized for assays.

2.6.3.2 Dosage of total soluble amino acids and proline

According to the method defined by Yemm and Cocking [25], amino acids were determined by the ninhydrin reaction. Amino acids undergo hot oxidative denaturation in the presence of ninhydrin with the release of CO_2, NH_3, and one aldehyde molecule. Ammonia reacts with the ninhydrin molecule in acetone and in the presence of KCN to form a violet-blue complex whose color intensity is proportional to the concentration of amino acids in the solution. The reaction medium consisted of 50 µL of extract; 0.5 mL of citrate buffer (0.2 M; pH: 5); 1 mL of 80° ethanol, and 0.5 mL of ninhydrin reagent (1% ninhydrin and 0.06% KCN in acetone). This medium was incubated at 100°C during 15 min. After icing the mixture, the absorbance

of the formed complex was read at 570 nm with a spectrophotometer (HACH DR3900). The amino acid content was calculated through reference to a calibration curve performed using pure glycine.

For proline, the optical density of the complex was provided at 440 nm. The proline concentration was calculated by reference to a standard curve made with pure proline.

2.6.3.3 Total soluble sugars

The evaluation of total soluble sugars was performed conferring to the Anthrone method [26]. In a concentrated, hot acidic medium, monosaccharides with at least 5 carbon atoms were dehydrated and transformed into furfuranic compounds. The latter are likely to combine with Anthrone to form green-colored complexes. The reaction mixture consisting of 0.5 mL of alcohol at 80°, 25 µL of extract, and 2.5 mL of Anthrone solution was hotness in a water bath (DAGLEF PATZ) at 80 °C during 20 min. After icing, the optical density of the green furfural complex solution obtained was read at 620 nm with a spectrophotometer (HACH DR3900). The content of total soluble sugars was calculated by reference to a standard curve made with glucose.

2.7 Data analysis

Data were firstly tested to analysis of variance (ANOVA) assumptions (the normality and the homogeneity of variance). Then, the data were performed through ANOVA using IBM SPSS Statistics 20 and R software version 4.0.5. The averages were classified by SNK test at 5% threshold. The Pearson's correlation test between variables and the illustrations were performed with R version 4.0.5 software.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Influence of water deficit and phosphate adjustment on growth of BG

The water deficit and phosphate fertilization significantly influenced the number of leaves and the height of BG (Table 2). The interaction "water deficit and P doses" was significant ($p < 0.001$) for both parameters. Overall, phosphate fertilization significantly increased the height ($p < 0.01$) and the N° of leaves ($p < 0.05$) of BG (Table 2). The water deficit significantly ($p < 0.01$) reduced the growth. Thus, after four weeks of water deficit, the plant height at 0 mg P_2O_5 at 60% FC (Field Capacity) significantly exceeded that at 0 mg P_2O_5 at 30% FC (Table 3). However, phosphate input mitigates the adversative influence of the water deprivation on BG growth. Thus, after four weeks of water deficit, the N° of leaves of treatment 100 mg P_2O_5 at 30% FC was not meaningfully ($p > 0.05$) different from that of 100 mg P_2O_5 at 90 % CC (Table 3).

Table 2. Probability (*P*) relative to the plant height and number of leaves of Bambara groundnut over time

Parameters	N° of leaves				Plant height (cm)			
Time (week)	2WAS	4WAS	6WAS	8WAS	2WAS	4WAS	6WAS	8WAS
Landrace (L)	**	**	**	***	**	**	***	***
Water deficit (WD)	ns	ns	ns	*	*	**	**	ns

P	ns	*	ns	ns	**	**	**	*
L*WD	*	ns	ns	ns	**	ns	ns	ns
L*P	ns	ns	ns	ns	ns	ns	ns	ns
WD*P	**	***	***	***	*	***	***	***
L*WD*P	*	**	ns	ns	ns	ns	ns	ns

, ** and *: significant at the 0.05, 0.01 and 0.001 levels, respectively, ns: no significant difference at the 0.05 level.*

Table 3. Effect of P-fertilization and water deficit on the plant height (y) and number of leaves (y) of Bambara groundnut over time (x in week)

Field capacity (%)	P (mg P_2O_5.kg^{-1} soil)	Regression equation of plant height (cm)	R^2	Regression equation of n° of leaves	R^2
30	0	$y = 1.23x + 16.88$	0.91	$y = 2.27x + 6.88$	0.99
	20	$y = 1.81x + 18.43$	0.98	$y = 2.06x + 6.21$	0.99
	40	$y = 1.87x + 18.10$	0.98	$y = 2.16x + 7.28$	0.99
	60	$y = 1.72x + 16.62$	0.99	$y = 1.92x + 7.43$	0.99
	100	$y = 1.96x + 17.98$	0.99	$y = 1.94x + 6.63$	0.99
60	0	$y = 1.74x + 18.16$	0.88	$y = 1.85x + 6.02$	0.99
	20	$y = 1.81x + 17.54$	0.92	$y = 1.78x + 7.40$	0.99
	40	$y = 1.25x + 19.47$	0.99	$y = 1.88x + 7.73$	0.98
	60	$y = 2.11x + 20.03$	0.84	$y = 1.66x + 6.57$	0.99
	100	$y = 1.50x + 17.22$	0.98	$y = 2.14x + 7.25$	0.98
90	0	$y = 1.59x + 17.03$	0.97	$y = 2.00x + 6.68$	0.99
	20	$y = 1.57x + 17.21$	0.98	$y = 2.01x + 7.43$	0.99
	40	$y = 2.28x + 18.36$	0.96	$y = 1.80x + 7.06$	0.99
	60	$y = 1.43x + 17.07$	0.99	$y = 2.00x + 6.87$	0.99
	100	$y = 1.91x + 17.80$	0.97	$y = 1.97x + 6.69$	0.99

x: four to eight weeks after sowing

3.1.2 Effect of water deprivation and P-fertilization on biomass and physiological traits

3.1.2.1 Biomass

Phosphate fertilization significantly improved the biomass of BG (Table 4). But the water deficit did not affect it. However, the interactions water deficit and phosphate fertilization were significant for root weight ($p < 0.001$), plant weight (total) ($p < 0.01$) and root/shoot ratio ($p < 0.05$). Thus, at the control (90% FC), the total dry weight augmented meaningfully ($p < 0.01$) by 27.3% from 0 to 100 mg of P_2O_5. The shoot dry weight augmented meaningfully ($p < 0.01$) by 22.6% from 0 to 100 mg P_2O^5 at 30% FC (Table 5).

Table 4. Probability relative to biomass, physiological and biochemical parameters

TRT	Shoot weight (g)	Root weight (g)	Plant weight (g)	Root/ shoot ratio	PWC (g.dw^{-1})	Leaf RWC (%)	Proline (µg. g^{-1}fw)	Sugar (mg. g^{-1}fw)	AA (µg. g^{-1}fw)	SRI
L	ns	***	***	***	***	***	***	***	ns	***
P	***	**	**	ns	ns	***	ns	***	**	**
WD	ns	ns	ns	ns	***	**	***	***	***	ns
L*P	ns	*	*	**	ns	**	ns	ns	ns	***
L*WD	ns	**	**	ns	ns	ns	***	*	ns	ns
P*WD	ns	***	**	*	ns	ns	ns	ns	ns	*
L*P*WD	**	ns	***	***	ns	***	ns	ns	ns	***

*L: landrace, WD: water deficit, P: phosphorus, RWC: relative water content, PWC: plant water content, AA: amino acid, SRI: stress resistance index, *, ** and ***: significant at the 0.05, 0.01 and 0.001 levels, respectively, ns: no significant difference at the 0.05 level.*

3.1.2.2 Water Content

The water deficit significantly decreased the water content of the plants, but the different doses of P_2O_5 did not affect it. Thus, compared to the control (90% FC), the water deficit significantly reduced ($p < 0.05$) the plant's water content by 20 and 37%, respectively, to 60 and 30% FC at 0 mg P_2O_5 (Table 5).

3.1.2.3 Relative Water Content

The water deficit significantly reduced the relative water content (RWC) of BG leaves (Table 4). Thus, at 0 mg of P_2O_5, RWC decreased significantly ($p < 0.001$) by 22% from 90 to 60% FC, and by 7% from 60 to 30 % FC. High levels of P_2O_5 increased the RWC of leaves under water deficit (Table 5).

3.1.2.4 Stress Resistance Index

The water deficit did not affect the stress resistance index of BG. But, P_2O_5 tended to improve this index significantly. Thus, at 60% FC, the highest resistance index (1.3) was observed at 40 mg P_2O_5 (Table 5).

3.1.3 Influence of water deprivation and P-fertilization on biochemical traits

3.1.3.1 Proline Content

The doses of P_2O_5 did not affect the proline concentration. But the water deprivation significantly ($p < 0.001$) influenced it. The interaction "water deficit and P_2O_5 dose" was significant ($p < 0.01$). The water deficit significantly increased ($p < 0.001$) the proline content of the BG leaves. Thus, at 0 mg of P_2O_5, the proline content at 30% FC was twice that of the control (90% FC) (Table 5).

3.1.3.2 Total Soluble Sugars Content

The water deficit and P_2O_5 doses significantly increased the soluble sugars content of BG leaves ($p < 0.001$) (Table 4). The optimum effect of P_2O_5 on the soluble sugar content was observed at 60 mg of P_2O_5. Thus, at 0 mg of P_2O_5, the total soluble sugars content of the leaves significantly ($p < 0.001$) increased by 5% at 60% FC, and 78% at 30% FC compared to the control (90% FC). At the control (90% FC), the doses 20, 40, 60, 100 mg of P significantly increased the sugar content by 39, 50, 65, and 47%, respectively, compared to 0 mg of P_2O_5 (Table 5)

Table 5. Effect of P-fertilization and water deficit on the biomass and biochemical content of Bambara groundnut

Water deficit (% FC)	P (mg P_2O_5.kg^{-1} soil)	Shoot weight (g)	Root weight (g)	Plant weight (g)	Root/ shoot ratio	Plant water content (g.dw^{-1})	Leaf RWC (%)	Proline content (µg.g^{-1} fw)	Sugar content (mg.g^{-1} fw)	AA content (µg.g^{-1} fw)	Stress resistance index
30	0	2.65^{ab}	3.02^{ab}	5.67^{ab}	1.33^{ab}	2.17^{a}	66.11^{a}	0.17^{b}	5.56^{bcd}	157.69^{b}	1.09^{ab}
	20	2.33^{a}	1.74^{a}	4.07^{a}	1.52^{ab}	3.45^{b}	59.57^{a}	0.147^{b}	5.96^{cd}	149.6^{ab}	0.86^{a}
	40	2.88^{ab}	1.99^{a}	4.87^{ab}	1.49^{ab}	2.34^{ab}	65.09^{a}	0.149^{b}	6.03^{cd}	160.45^{b}	1.18^{ab}
	60	3^{ab}	1.52^{a}	4.52^{ab}	2.29^{b}	2.72^{ab}	72.58^{ab}	0.112^{ab}	6.52^{d}	147.25^{ab}	1^{ab}
	100	3.25^{b}	2.23^{a}	5.48^{ab}	1.77^{ab}	2.62^{ab}	73.94^{ab}	0.159^{b}	5.69^{bcd}	149.43^{ab}	1.04^{ab}
60	0	2.6^{ab}	2.31^{ab}	4.91^{ab}	1.54^{ab}	2.75^{ab}	71.05^{ab}	0.091^{ab}	3.28^{a}	144.09^{a}	0.95^{ab}
	20	2.69^{ab}	2.16^{a}	4.84^{ab}	1.37^{ab}	2.8^{ab}	76.07^{ab}	0.06^{a}	4.52^{bc}	138.96^{ab}	1.11^{ab}
	40	2.98^{ab}	2.62^{ab}	5.6^{ab}	1.6^{ab}	2.62^{ab}	76.81^{ab}	0.099^{ab}	4.98^{bc}	150.94^{ab}	1.3^{b}
	60	2.8^{ab}	2.45^{ab}	5.25^{ab}	1.18^{a}	2.69^{ab}	75.82^{ab}	0.067^{ab}	5.24^{bcd}	134.67^{ab}	1.19^{ab}
	100	2.81^{ab}	2.14^{a}	4.96^{ab}	1.77^{ab}	3.21^{ab}	77.31^{ab}	0.073^{ab}	4.78^{bc}	122.98^{a}	0.91^{ab}
90	0	2.72^{ab}	2.62^{ab}	5.3^{ab}	1.35^{ab}	3.45^{b}	91.06^{b}	0.083^{ab}	3.12^{a}	139.02^{ab}	
	20	2.43^{ab}	2.3^{ab}	4.73^{ab}	1.68^{ab}	2.85^{ab}	72.35^{ab}	0.079^{ab}	4.33^{b}	131.74^{ab}	
	40	2.69^{ab}	1.55^{a}	4.24^{ab}	1.96^{ab}	3.01^{ab}	76.95^{ab}	0.065^{a}	4.67^{bc}	143.9^{ab}	
	60	2.74^{ab}	1.79^{a}	4.53^{ab}	1.76^{ab}	2.96^{ab}	77.91^{ab}	0.093^{ab}	5.16^{bcd}	130.39^{ab}	
	100	3.1^{ab}	3.7^{b}	6.8^{b}	1.22^{ab}	3.13^{ab}	76.47^{ab}	0.092^{ab}	4.6^{bc}	130.25^{ab}	

RWC: Relative Water Content, AA: amino acid. Means followed by the same letter in a column are not significantly different at the 5 % threshold.

3.1.3.3 Total Amino Acid Content

Overall, the water deficit significantly ($p < 0.001$) increased the leaves' total amino acid content. Thus, at 0 mg P_2O_5, the amino acid content at 30% FC significantly exceeds those at 60 and 90% FC (Table 5). The effect of doses of P_2O_5 on the total amino acid content was hardly noticeable.

3.1.4. Influence of landrace on growth, physiological and biochemical traits

3.1.4.1 Growth

The dry shoot biomass did not vary significantly with landraces (Fig. 2). Landraces L2 and L3 were meaningfully ($p < 0.001$) grander (plant height) than L1; but L1 and L4 had a meaningfully ($p < 0.001$) higher N° of leaves than L2 (Table 6). L3 and L2 produced meaningfully ($p < 0.001$) higher dry biomass (roots and whole plant) than L1 (Fig. 2). However, L1 significantly showed the best root/shoot ratio (dry biomass).

Table 6. Effect of landrace on the growth (y) of Bambara groundnut over time (x in week)

Landraces	Regression equation of plant height (cm)	R^2	Regression equation of N° leaves	R^2
L1	$y = 18.50x^{0.17x}$ (a)	0.99	$y = 2.07x + 7.02$ (b)	0.99
L2	$y = 19.83x^{0.18x}$ (b)	0.99	$y = 1.88x + 6.69$ (a)	0.99
L3	$y = 19.41x^{0.17x}$ (b)	0.99	$y = 1.94x + 7.10$ (b)	0.99

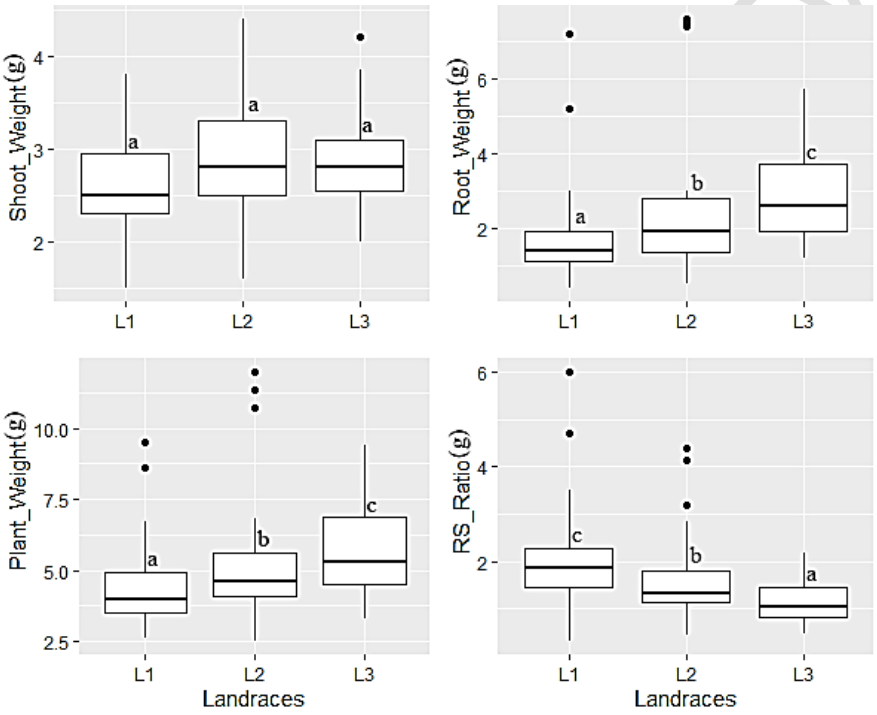

Fig. 2 Effect of landrace on the biomass of Bambara groundnut (n=45).
L1: ivory cream seed coat, L2: red seed coat, L3: ivory cream seed coat with grey eyes, RS: Root/Shoot. Means followed by the same letter in a column are not significantly different at the 5% threshold.

3.1.4.2 Physiological traits

Landraces L1 and L2 had a meaningfully ($p < 0.001$) greater plant water content than L3 (Fig. 3). The relative water content of the leaves was highest at L2 (Fig. 3). Yet, L3 had the highest stress resistance index (Fig. 4).

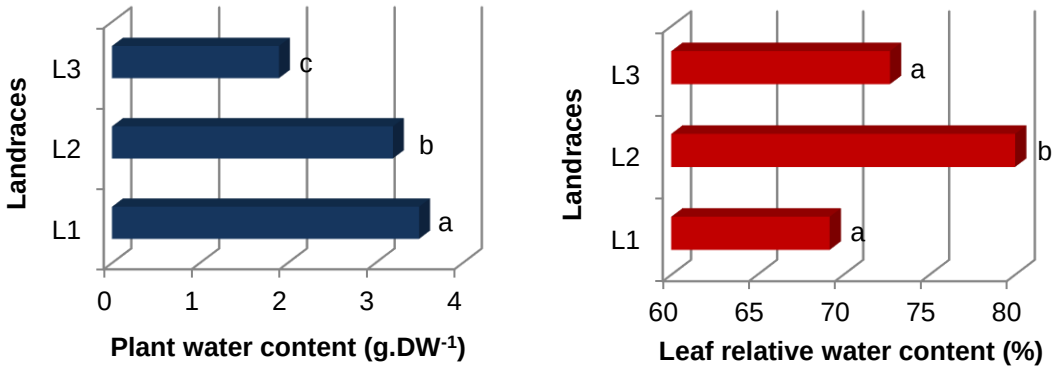

Fig. 3. Effect of landrace on physiological traits of Bambara groundnut (n=45).
L1: ivory cream seed coat, L2: red seed coat, L3: ivory cream seed coat with grey eyes, Means followed by the same letter in a column are not significantly different at the 5% threshold.

3.1.4.3 Biochemical traits

Landraces L3 had the highest total soluble sugar content. Yet, the proline content was highest at L2 (Fig. 4). The landrace did not affect the total amino acid content of the leaves of BG (Fig. 4).

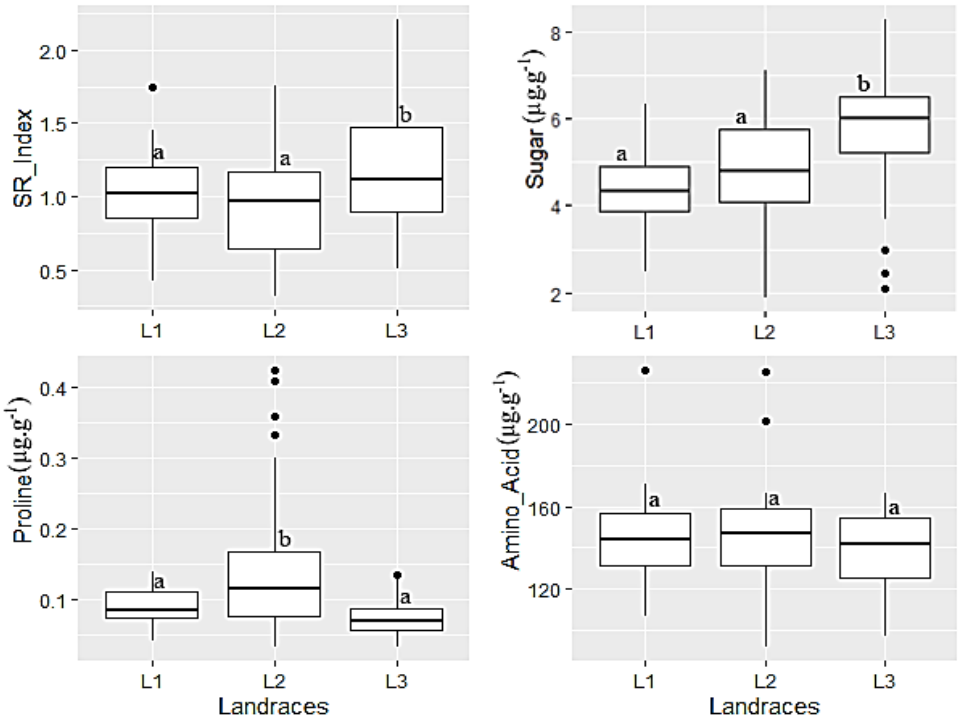

Fig. 4. Effect of landrace on stress resistance index and biochemical content of Bambara groundnut (n=45).
L1: ivory cream seed coat, L2: red seed coat, L3: ivory cream seed coat with grey eyes, SR index: stress resistance index. Means followed by the same letter in a column are not significantly different at the 5% threshold.

3.1.5 Correlation

Plant water content was negatively and significantly correlated with total soluble sugar concentration (r = -0.193*), shoot dry weight (r = -0.343***), root dry weight (r = -0.484***) and plant dry weight (r = 0.505***) (Fig. 5). However, it was positively and meaningfully correlated with root/shoot ratio (r = 0.405***) (Fig. 5). Proline concentration was positively and meaningfully correlated with total soluble sugar concentration (r = 0.213*), total soluble amino acid (r = 0.363*) (Fig. 5). Significant and negatively correlation was found among leaf's relative water content and total soluble sugar content (r = -0.187*) (Fig. 5).

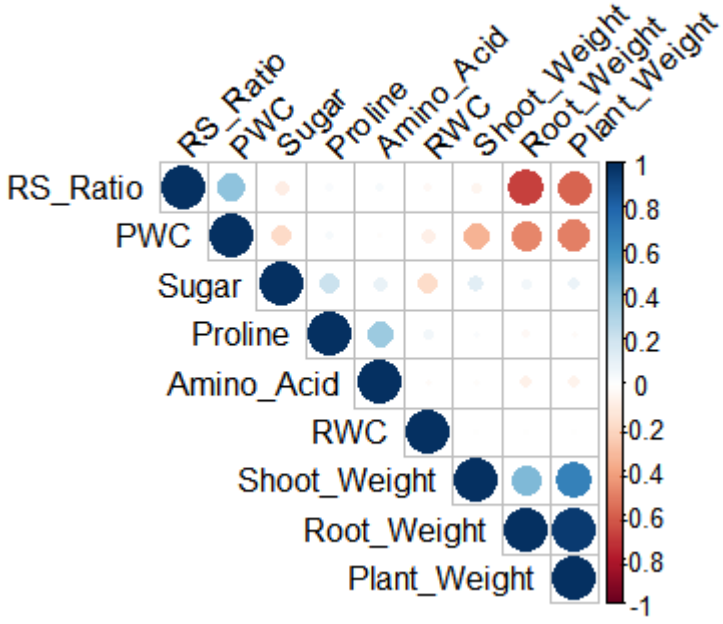

Fig. 5. Pearson correlation matrix among evaluated variables.
RS: Root/Shoot, RWC: relative water content in leaf, PWC: plant water content. Positive correlations were displayed in blue and negative correlations in red. The correlogram represents the correlations for all pairs of variables. The intensity of the color was proportional to the correlation coefficient so the stronger the correlation (i.e., the closer to -1 or 1). The color legend on the right-hand side of the correlogram shows the correlation coefficients and the corresponding colors.

3.2 Discussion

3.2.1 Growth

Water stress significantly decreased the growth (height and N° of leaves) of BG but not the biomass (shoot weight, root weight, plant weight). However, P-fertilization significantly increased the Bambara-bean's biomass and growth. It mitigates the adversative impact of the water stress on growth. This improvement in growth may be due to the different P-doses applied [27]. Indeed, the vegetative development is very disturbed under limiting water supply conditions. P stimulates root development and increases the root's biomass, favoring nutrition and plant growth [28, 29]. This result corroborates those of [30] on rapeseed. [21] also found similar results on soy.

3.2.2 Physiological parameters

The water deficit significantly decreased the PWC and the RWC. The P-doses did not affect PWC but enhanced RWC and the stress resistance index. The water deficit did not affect the stress resistance index. Increased water in plant leaf tissues from P-elevated substrates could stimulate transportation and solute build-up for enhanced growing. This outcome is comparable to those of [31] on cotton. During the sampling, they noted by visual observation and leaf handling that, the plants in the low P-substrates have coarser and drier leaf than the fresh and turgid leaf of the plants of the substrates with high P-content. These results are also consistent with those of [32], who stated that cell membrane steadiness, osmoregulation, and water deficit resistance augmented with P-fertilization in corn leaf under water deficit.

3.2.3 Biochemical traits

The P-doses did not affect the proline concentration of the leaves. But the water deficit significantly increased it. The water deficit and P-doses significantly increased the sugars and the amino acids content of leaves. Globally, the P-doses and water stress significantly increased the build-up of solutes (sugars, proline, and amino acids). The contribution of P would have promoted the use quantity of solutes in the roots. These modifications would have led to an upsurge in the acquirement of P and other nutrients [6] through the formation of a vigorous root system that provides a higher tolerance to adverse conditions. Increasing P-doses increases P-uptake in low FC treatments. This increase would lead to an increase in the provision of osmotically active assimilates to cells in growing leaves. This finding is analogous with those of [33] on *Alnus cremastogyne*.

3.2.4 Landrace

The shoot biomass and the amino acids content did not vary significantly with landraces. But landrace influenced plant height, N° of leaves, biomass (root, plant weight, and root/shoot ratio), PWC, RWC, stress resistance index and the concentrations of proline and sugars in leaves. The landraces studied showed different adaptation strategies to water deficit. L3 had a high leaf number, plant height, biomass, leaf sugar content and SRI. Whereas L1, despite its high leaf number, showed reduced plant height and biomass, but had a high R/S ratio and therefore the ability to increase its root biomass under water deficit for better plant water supply. Hence its high plant water content. L2 had a reduced number of leaves, but high plant height, biomass, R/S ratio, PWC, RWC and proline content. L2, despite having a high R/S ratio and increased proline accumulation in its leaves, showed the lowest SRI due to maintaining high biomass, RWC and PWC under water deficit. These results could be explicated through the presence of a genotypic dissimilarity between these landraces [34-37].

3.2.5 Correlation

PWC was negatively and significantly correlated with sugars content, root weight and plant weight. It was positively and significantly correlated with root/shoot ratio and RWC. The negative correlation between PWC and biomass would be explained by the preferential allocation of plant resources to the roots, thus favoring its growth at the expense of the vegetative part [6]. For this reason, the correlation between PWC and root/stem ratio was rather positive. Proline content was positively and significantly correlated with sugars and amino acids contents. The increased accumulation of solutes is an adaptation strategy of plants to water deficit. Indeed, plants dynamically reduce their osmotic potential to support cell growth and evade cell harm caused by dehydration during water deficit by accruing additional solutes. These metabolites are vital in upholding osmotic balance and shielding

macromolecules, and membranes, thus providing resistance to water deprivation and cellular dehydration [32].

4. CONCLUSION

This study shows that the water deficit significantly reduces Bambara-bean growth. Still, it induces a strong accumulation of amino acids, soluble sugars, and proline concentrations. P-fertilization significantly enhances the growth and biochemical composition under water deficit. It also promotes good maintenance of physiological parameters. Adequate P-nutrition helps maintain a higher percentage of dry matter and alleviates the adversative influence of water stress on Bambara-bean. Therefore, a sufficient P-supply is recommended to help plants escape, avoid or tolerate the water deficit. L2 and L3 seem more tolerant to stress than L1 at this growth stage.
(Please add experiment photographs appropriately)

REFERENCES

[1] Musa M, Massawz F, Mayes S, Alshareef I, Singh A. Nitrogen fixation and N-balance studies on Bambara groundnut (*Vigna subterranea* L. Verdc) landraces grown on tropical acidic soils of Malaysia. Commun Soil Sci Plant Anal. 2016;47(4). doi: 10.1080/00103624.2016.1141928.

[2] Begemann F. Ecogeographic differentiation of Bambara groundnut (*Vigna subterranea*) in the collection of the International Institute of Tropical Agriculture (IITA). Niederkleen: Wissenschaftlicher Fachverlag Dr Fleck;1988.

[3] Temegne NC, Gouertoumbo WF, Wakem G-A, Nkou Foh TD, Youmbi E, Ntsomboh-Ntsefong G. Origin and ecology of Bambara groundnut (*Vigna subterranea* (L.) Verdc: A Review. JENR. 2018;2(4):1–10. doi: 10.23880/jenr-16000140

[4] Temegne NC, Dooh JPN, Nbendah P, Ntsomboh-Ntsefong G, Taffouo VD, Youmbi E. Cultivation and utilization of Bambara groundnut (*Vigna subterranea* (L.) Verdc.), a neglected plant in Cameroon. Asian Plant Res J. 2020;4(2):9–21. doi: 10.9734/APRJ/2020/v4i230081

[5] Hassan M, Uddin MK, Mohammed MTM, Zuan ATK, Motmainna M. Growth, yield, nodulation and amino acid content of Bambara groundnut (*Vigna subterranea*) under inorganic and organic fertilizer application. Legume Res. 2021;44(3):322–327. doi: 10.18805/LR-576.

[6] Tsoata E, Temegne CN, Youmbi E. Analysis of early biochemical criterion to screen four Fabaceae plants for their tolerance to drought stress. Int J Curr Res. 2017;9(1):44568–44575.

[7] Temegne NC. Improvement in the performances of Voandzou (*Vigna subterranea* (L.) Verdc.) in response to phosphate deficiency through chemical and biological fertilization. PhD Thesis. Yaounde: University of Yaounde I; 2018.

[8] Musah M, Azeh Y, Mathew JT, Nwakife CN, Mohammed AI, Saidu F. Nutritional evaluation of Bambara groundnut (*Vigna subterranea* (L.) Verdc) from Lapai, Nigeria. Afr J Agric Food Sci. 2021;4(4):32–39. doi: 10.52589/AJAFS_SQI5U7CN

[9] FAOSTAT. Production statistics. United Nations Food and Agriculture Organization, Rome; 2024. https://www.fao.org/faostat/fr/#data/QCL. Accessed on 9 May 2024.

[10] Temegne NC, Tsoata E, Ntsomboh-Ntsefong G, Agendia AP, Ngome FE, Youmbi E. Mechanisms and approaches of enhancing drought stress tolerance in crop plants. In: Hasanuzzaman M (ed.). Climate-resilient agriculture, Cham: Springer; 2023. doi:10.1007/978-3-031-37428-9_19.

[11] Temegne NC, Mbogne TJ, Nbendah P, Youmbi E, Taffouo VD, Ntsomboh-Ntsefong G. Effect of phosphate deficiency on growth and phosphorus content of three Voandzou (*Vigna subterranea* (L.) Verdc.) varieties. IOSR J Agric Vet Sci. 2015;8(9):52–59. doi: 10.9790/2380-08915259.

[12] Tsoata E, Temegne NC, Youmbi E. Analysis of early physiological criteria to screen four Fabaceae plants for their tolerance to water stress. Int J Recent Sci Res. 2016;7(11):14334–14338.

[13] Tsoata E, Temegne CN, Youmbi E. Analysis of early growth criterion to screen four Fabaceae plants for their tolerance to drought stress. RJLBPCS. 2017;2(5):94–109.

[14] Temegne NC, Nkou Foh TD, Taffouo VD, Wakem G-A, Youmbi E. Effect of mycorrhization and soluble phosphate on growth and phosphorus supply of Voandzou (*Vigna subterranea* (L.) Verdc.). Legume Res. 2018;41(6):879–884. doi: 10.18805/LR-388.

[15] IRAD. Contribution of research to improving the production and consumption of food legumes in Cameroon. Yaounde: IRAD (Institute of Agricultural Research for Development); 2013.

[16] Temegne NC, Nkou Foh TD, Taffouo VD, Ntsomboh-Ntsefong G, Youmbi E. Influence of mycorrhization and phosphate fertilizer on growth of Voandzou (*Vigna subterranea* (L.) Verdc.). Int J Biol Chem Sci. 2017;1(6):2587–2593. doi: 10.4314/ijbcs.v11i6.3

[17] Temegne NC, Wakem G-A, Taffouo VD, Mbogne TJ, Onguene AN, Youmbi E, Ntsomboh-N G. Effect of phosphorus fertilization on arbuscular mycorrhizal fungi in the Bambara groundnut rhizosphere. Afr J Microbiol Res. 2017;11(37):1399–1410. doi: 10.5897/AJMR2017.8680.

[18] Iheanacho NS, Lawrence FS, Oluwole AB. Effects of induced water stress at the flowering stage of Bambara groundnut (*Vigna subterranea* L. Verdc) on its yield. Am J Biosci. 2021;9(6):192–198. doi: 10.1081/PLN-120002969.

[19] Vance CP, Uhde-Stone C, Allan DL. Phosphorus acquisition and use: Critical adaptations by plants for securing a nonrenewable resource. New Phytol. 2003;157:423–447. doi: 10.1046/j.1469-8137.2003.00695.x.

[20] Syers JK, Johnston AE, Curtin D. Efficiency of soil and fertilizer phosphorus use - reconciling changing concepts of soil phosphorus behaviour with agronomic information. Rome: FAO; 2008.

[21] Rotaru V. The effects of phosphorus application on soybean plants under suboptimal moisture conditions. Lucrări Ştiinţifice. 2010;53(2):27–30.

[22] Guissou T, Ba AM, Plenchette C, Guinko S, Duponnois R. Effects of arbuscular mycorrhizae on tolerance to water stress in four fruit trees: *Balanites aegyptiaca* (L.) Del., *Parkia biglobosa* (Jacq.) Benth., *Tamarindus indica* L. and *Zizyphus mauritania* Lam. Sécheresse. 2001;12(2):121–127.

[23] Clarke JM, McCaig TN. Excised leaf water relation capability as an indicator of drought resistance of *Triticum* genotypes. Can J Plant Sci. 1982;62(3):571–576. doi: 10.4141/cjps82-086.

[24] Fischer RA, Maurer R. Drought resistance in spring wheat cultivars. Grain yield responses. Aust J Agric Res. 1978;29:897–903.

[25] Yemm EM, Cocking EC. 1955. The determination of amino acids with ninhydrin. The Analyst. 80:209–213. doi: 10.1039/AN9558000209.

[26] Yemm EM, Willis AJ. 1954. The estimation of carbohydrates in plants extracts by anthrone. Biochem J. 57(3):508–514. doi: 10.1042/bj0570508.

[27] Temegne NC, Taffouo VD, Tadoh TC, Gouertoumbo WF, Wakem G-A, Nkou Foh TD, Kenmogne NPP, Youmbi E. Effect of phosphate fertilization on growth, yield and seed phosphorus content of Bambara pea (Vigna subterranea) landraces. J Anim Plant Sci. 2019;29(3):703–713.

[28] Temegne C, Ngome AF, Fotso KA. Effect of soil chemical composition on nutrient uptake and yield of cassava (*Manihot esculenta* Crantz) in two agro-ecological zones of Cameroon. Int J Biol Chem Sci. 2015;9(6),2776-2788. doi: 10.4314/ijbcs.v9i6.21.

[29] Temegne CN, Ngome FA. Fertility management for cassava production in the Centre Region of Cameroon. J Exp Agric Int. 2017;16(5):1–8. doi: 10.9734/JEAI/2017/34111.

[30] Said-Al Ahl HAH, Mehanna HM, Ramadan MF. Impact of water regime and phosphorus fertilization and their interaction on the characteristics of rapeseed (*Brassica napus*) and fatty acid profile of extracted oil. Comm Biometry Crop Sci. 2016;11:64–76.

[31] Singh V, Pallaghy CK, Singh D. Phosphorus nutrition and tolerance of cotton to water stress II. Water relations, free and bound water and leaf expansion rate. Field Crops Res. 2006;96(2–3):199–206. doi: 10.1016/j.fcr.2005.06.011.

[32] Premchandra GS, Saneoka H, Fujita K, Ogata S. Cell membrane stability and leaf water relations as affected by phosphorus nutrition under water stress in maize. Soil Sci Plant Nutr. 1990;36:661-666. doi: 10.1080/00380768.1990.10416803.

[33] Tariq A, Pan K, Olatunji OA, Graciano C, Li Z, Sun F, Zhang L, Wu X, Chen W, Song D, Huang D, Xue T, Zhang A. Phosphorous fertilization alleviates drought effects on *Alnus cremastogyne* by regulating its antioxidant and osmotic potential. Sci Rep. 2018;8:5644. doi:10.1038/s41598-018-24038-2

[34] Kamtchoum SM, Nchoutnji I, Temegne CN, Tonfack LB, Fofe L, Seutchueng TGT, Kemayou CM, Notche FK, Suh C, Youmbi E. Comparative effect of biological fixation of nitrogen and chemical fertilizer on yield optimization of two sorghum varieties in the Western Highlands. Asian J Agric Hortic Res. 2019;4(3):1–10. doi: 10.9734/ajahr/2019/v4i330023

[35] Kamtchoum SM, Nuemsi PPK, Tonfack LB, Edinguele DGM, Kouahou WN, Youmbi E, Temegne CN. Production of bean (*Phaseolus vulgaris* L.) under organo-mineral fertilization in humid forest Agro-ecological zone with bimodal rainfall pattern in Cameroon. Ann Res Rev Biol. 2018;29(4):1–11. doi: 10.9734/ARRB/2018/44607.

[36] Nuemsi PPK, Tonfack LB, Taboula JM, Mir BA, Mbanga MRB, Ntsefong GN, Temegne CN, Youmbi E. Cultivation systems using vegetation cover improves sustainable production and nutritional quality of new rice for Africa in the tropics. Rice Sci. 2018;25(5):286–292. doi: 10.1016/j.rsci.2018.08.003.

[37] Temegne NC, Tsoata E, Ngome AFE, Tonfack LB, Agendia AP, Youmbi E. Lima bean. In: Pratab A, Gupta S. (eds). The beans and the peas - From orphan to mainstream crops. Elsevier; 2020. doi: 10.1016/B978-0-12-821450-3.00009-3.