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Original Research Article

Evaluation of Some Niger Morphotypes of Voandzou (*Vigna subterranea* L. Verdc.) in early stress conditions

Daouda Ousmane S[1*], Harouna Issa A[2], Aghali Alhassane[2], Baragé M[3]
[1]Laboratory of Biotechnology and Plant Breeding, Institute of Radioisotopes, University Abdou Moumouni, Niamey Niger
[2]Genetics & Plant Breeding Department of Biology; Faculty of Sciences and Technology
[3]Department of Plant Production; Faculty of Agronomy University Abdou Moumouni Niamey, Niger

***Corresponding author**
Daouda Ousmane S
Email: dsaniri@yahoo.fr

Abstract: Bambara Groundnut (BGN) (Vigna subterranea (L.) Verdc.) Could be of great importance in the semi-arid Sahel, for its nutritional quality and its ability to fertilize the soil. Unfortunately, yields were very low due to the erratic rainfall pattern in the Sahelian regions. That is why a study is conducted to determine the effect of early drought on the growth of BGN seedling was studied in semi-controlled-environment in semi-opened greenhouses in Niger. There were twelve morphotypes coming from a collection in 2013 from producers in the Western and Central areas of Niger country and five watering regimes, expressed in % of field capacity, ranging from fully irrigated to strictly dry pot, were experimented : W1=100%, W2=75%, W3=50%, W4=37.5%; W5=25%, and W6 with no irrigation after sowing. Drought caused significant reductions in all growth parameters. The morphotypes differed significantly in their response to drought; Morphotype NE-04 exhibited the best drought tolerance with respectively 6.8% and 16.7% decrease in fresh weight and number of leaves, and the best Drought Susceptibility Index of 0.94. We suggest that this promising line to be considered by breeders, with an improvement in its performance especially its low germination percentage.
Keywords: Vigna subterranea, Drought Stress, Niger, Biodiversity

INTRODUCTION

Bambara groundnut (Vigna subterranea L. Verde.) is grown as a subsistence crop in semi-arid regions of Africa where the success of other legumes is uncertain because of poor soils, drought and disease. However, previous research on the crop has been limited, largely because its commercial value is restricted by low lipid content, below 8% [1], even though it is an important source of protein at the subsistence level. Bambara groundnut has the status of neglected and underutilized crop species (NUCS), as defined by Chivenge *et al.;* [2], which can have great potential to reduce food and nutrition insecurity, particularly for resource poor households in Sub-Saharan Africa [3]. Neglected underutilized crops have been reported to have possibly evolved to become drought tolerant due to years of cultivation under often severe conditions.

Bambara groundnut seeds are a completely balanced food [4, 5]. It can be of great importance for the Sahel [6], whose diet is based mainly on cereals [7, 8, 5]. This nutritional quality is associated with its ability to fertilize the soil [9, 10]. Despite this importance, many factors limit the productivity of Voandzou. The yields of Bambara groundnut are extremely low and variable because the environments in which it is normally grown are characterized by various biotic and abiotic stresses. Currently, Bambara groundnut yields vary from 400 to 450 kg per hectare [11] for a potential of up to 3500 kg per hectare [12, 13]. The reasons were not been identified due to the limited research carried out on the crop in sub-Saharan Africa. However, Bambara groundnut is mainly grown by female [14, 15], generally in border bad quality areas. Besides that, due to the erratic rainfall pattern in the Sahelian regions, the first rain suitable for planting is often followed by several dry days that cause the failing of the planting and requires the farmers to replant [16]. The low yields have been associated with poor germination rate and variable germination rates which lead to poor crop establishment in the dry regions where the crop is grown [17, 18]. So that, providing producers with early drought tolerance Bambara groundnut lines will increase substantially farmers' income and contribute to food safety in the African Sahel. Working on a dozen of morphotypes of Niger, this work evaluated the genotypic variability of Vigna subterranea in seedling growth phase, identifying most drought tolerant lines.

MATERIAL AND METHODS

Seeds description

The plant material consisted of 12 morphotypes, called NE-01 to NE-14; coming from a collection in 2013 from producers in the Western and Central areas of Niger country. Morphotypes were described according to seed coat and eye color and pattern (Table 1). The weight of 100 seeds was determined according to the following procedure: Ten samples of seeds were weighed (approximately close to 100 g); then the number of inside each sample was counted and reported to 100 g. The seed moisture content was determined by the gravimetric method over 5 seeds dried in a ventilated oven at 105°C up to 5 days. Laboratory and field experiments were done during the dry season of 2014, at the Radio-Isotopes Institute (IRI) research station, University Abdou Moumouni of Niamey. The mean seasonal temperature was 28°C, relative humidity was very low (30%), with a day's length of 12.6 Hours.

Seed Germination

In sterile conditions

Seeds were surface sterilized for ten minutes in 10% sodium hypochlorite, than with thirty seconds 70% ethanol soaking followed by three rinses with distilled water. Sterilized seeds were placed in Petri dishes (90 mm diameter) containing 2 grams of sterile cotton soaked in 15 ml of sterile water. The Petri dishes were arranged in a completely randomized design consisting of twelve ecotypes and eight replicates. Each Petri dish received 5 surface sterilized seeds before being sealed with parafilm. All operations took place under laminar flow hood, in sterile conditions. Germination took place in dark, at 30°C in incubator. The germination is observed when radicle is visible to the naked eye (2-5 mm). Germination was followed for 7 days after sowing, by counting the number of seeds germinating each day, and total germination percentage was calculated.

In non-sterile conditions

To see the effect of sterilization and the impact of infection on the rate of Bambara groundnut seed germination, a second experiment in non-sterile conditions was conducted. The same experience without surface sterilization of the seeds was repeated with 3 replicates.

Early Seedling growth

Germinated seeds were used for the second experiment. A total of 192 PVC pots (25 cl) filled with a composite growth medium (mixture of topsoil with compost at a rate of 1: 1 (v/v)). Each pot received 150 g. Fives water regimes, expressed in % of field capacity, ranging from fully irrigated to strictly dry pot, were experimented : W1=100%, W2=75%, W3=50%, W4=37.5%; W5=25%, and W6 with no irrigation after sowing. Before sowing, each treatment receives the amount of water required to reach the corresponding humidity level. One germinated seed was sown by pot, with respect to gravitropism (root down), at 1-2 cm deep. There are four replicates pots per treatment. After sowing, each day, the water lost before was replaced in each pot. This is done by monitoring weight of each pot. Watering was made at the daily rate, the first week after planting and every two days until harvest. Likewise, seedling growth (number of leaves) was observed at the daily rate, the first week after planting and every two days until harvest (32 days after sowing). At harvest seedling length so as leaf size were measured with ruler, and then fresh biomasses were separated into root and shoot. After been rolled in a blotting paper to remove excess water, shoot and root fresh weights were measured using a precision balance.

The drought-susceptibility index (DSI)

The drought susceptibility index is a measure of plant drought tolerance in terms of minimization of the reduction of seedling growth caused by water stress. It was calculated according to Fernandez (19) for each genotype:

$$\mathrm{DSI} = \mathrm{Y_I Y_s} / \overline{\mathrm{Y}}_\mathrm{I}^{\,2}$$

Where Ys is the yield of cultivar under stress, Y_I the yield of cultivar under irrigated conditions, and $\overline{Y}_S$ and $\overline{Y}_I$ the mean yields of all cultivars under stress and non-stress conditions respectively.

DATA ANALYSIS

The experiment was carried out in a completely randomized design with three or four replicates, depending on the experimentation. Data were submitted to analysis of variance (ANOVA) to detect significant differences between means, with GENSTAT 12.1 version. Means differing significantly were compared using Newman-Keuls multiple range test at the 5% probability level.

RESULTS

Seeds characterization

The seed coat color and type of eyes are also very variable. Four base colors are dominant, with uniform or dotted styles: Cream, red, black and brawn. The weight of 100 seeds varied between 49.7 and 80.5 g, with an average value of 60.86 g. A multiple comparison ANOVA according Newman-Keuls (SNK), provides five distinct groups, with significant difference. Morphotype NE-04 has the highest weight. Seed water content varied from 5.72% to 8.52%, for the morphotypes NE-07 and NE-14, respectively. However there was no significant difference between morphotypes for seed water content.

Table 1: Morphotypes description relative to seeds characteristics (coat and eyes color)

Morphotype	100 seeds weight (g)	Seed Water Content (%)	seed coat color	color and shape of the eye
Ne -02	57.80^{ef}	5.93	Black uniform	No eye
Ne -03	51.67^{gh}	6.38	Cream	Eye as a thin cycle around hilum
Ne -04	80.54^{a}	7.46	Cream red striped	Large black eye
Ne -05	64.97^{d}	8.01	Cream spotted with black)	Eye as tick lines
Ne -06	68.61^{c}	6.27	Red dotted	No eye
Ne -07	57.78^{ef}	5.72	Brown uniform	No eye
Ne -09	60.69^{e}	6.81	Cream uniform	Eye as two tick lines
Ne -10	57.61^{ef}	6.80	Red uniform	No eye
Ne -11	60.87^{de}	7.04	Black rhomboid	No eye
Ne -12	53.04^{fgh}	6.15	Light red	No eye
Ne -13	55.73^{efg}	6.73	Light black	No eye
Ne -14	72.16^{b}	8.52	Cream lightly striped	Eye like butterfly
Average	**60.86**	**6.79**		
SD	**9.07**	**0.80**		

In vitro Germination

Germination percentages of Bambara groundnut morphotypes are presented in Table 2. The germination rate is relatively low. The average over all morphotypes is 66.7% and 64.8% respectively in non-sterile conditions (NSC) and sterile conditions (SC). Statistical analysis revealed a significant difference between morphotypes (Pr <0.002 for CNS and Pr <0.001 CS). However, the sterilization of seeds, as carried out in this experiment, did not affect the rate of germination. The classification of the morphotypes remains generally the same in both situations. The highest germination percentages were recorded with NE-09 and NE07 and the lowest with NE-04.

Table 2: In vitro germination of Bambara Groundnut in sterile (SC) and non-sterile conditions (NSC)

Morphotypes	NSC	SC
NE-02	80.0^{ab}	67.5^{a}
NE-03	66.7^{ab}	72.5^{a}
NE-04	46.7^{ab}	37.5^{b}
NE-05	60.0^{ab}	62.5^{ab}
NE-06	40.0^{b}	62.5^{a}
NE-07	86.7^{a}	75.0^{a}
NE-09	86.7^{a}	75.0^{a}
NE-10	46.7^{ab}	52.5^{ab}
NE-11	60.0^{ab}	52.5^{ab}
NE-12	80.0^{ab}	77.5^{a}
NE-13	66.7^{ab}	70.0^{a}
NE-14	80.0^{ab}	70.0^{a}
Average	**66.7**	**64.8**
SD	**15.6**	**11.3**

Means with the same letters in the same column are not significantly different at 5% level (Newman-Keuls multiple range test).

Effect of water stress on Seedling growth

Seedling growth has been observed for 32 days after sowing. The result showed two phases of growth: a rapid growth phase of one week duration where the effect of water stress is not perceptible; a second slower phase with a progressive flattening of the slope with the stress intensity (Figure 1).

All growth parameters, leaf number, plant height, leaf size, shoot and root fresh weights, were affected by various water regimes. Results showed that the seedling growth is significantly affected for W4 and W5 water regimes, when the soil water falls below 37.5%. The W2 water regime (75% FC) was the most suitable water conditions for Bambara groundnut growth, with highest significant values of all growth parameters (Table 2).

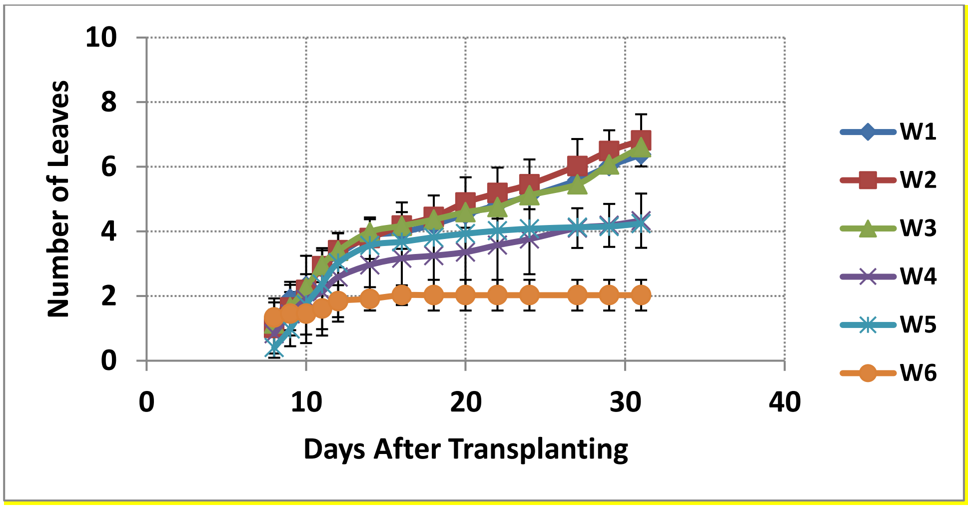

Fig 1: Bambara groundnut seedling growth as affected by soil water available expressed as field capacity percentage: W1=100% Field capacity (irrigation without limitation), W2=75%; W3=50%; W4=37.5%; W5=25% and W6=no irrigation after sowing (extremely dry).

Leaf size increase linearly, with the increase of soil water available, at an average rate of 3.22 10^{-2} cm each % of field capacity (%FC) (R^2 = 0.94), between 25% and 75% (figure 2). Corresponding values for shoot height and shoot fresh weight were 6.97 10^{-2} cm (R^2=0.79) and 2.53 10^{-2} g (R^2=0.87) respectively (Figure 3 and Figure 5). On the other hand, the fresh root weight adjustment follows a logarithmic function (Figure 4). The maximum root development occurs around 50%FC giving the highest value of 0.95.

Table 2: Effect of soil water regime on Bambara groundnut seedling growth

Morphotype	**Leaf Number**	**Plant height (cm)**	**Leaf size (cm)**	**Shoot Fresh weight (g)**	**Root Fresh weight (g)**
W1	6,3^a	14,2^a	5,7^a	1,720^b	1,103^a
W2	6,7^a	14,0^a	5,8^a	1,921^a	1,256^a
W3	6,4^a	13,4^a	5,2^b	1,684^b	1,259^a
W4	4,4^b	11,5^b	4,6^c	0,845^c	0,629^b
W5	4,2^b		4,0^d	0,671^d	

Means with the same letters in the same column are not significantly different at 5% level (Newman-Keuls multiple range test).

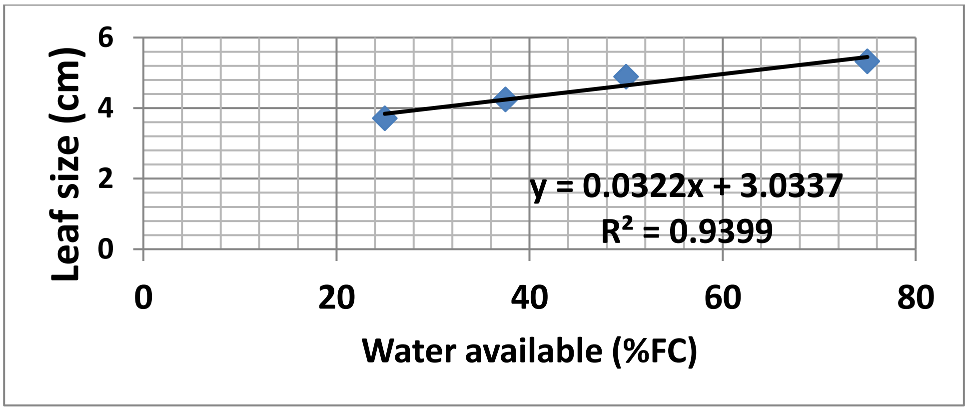

Fig 2: Relationships between leaf size and soil water available of Bambara groundnuts exposed to progressive soil drying at early seedling growing stage. Each point represents the average of 12 values, corresponding to 12 morphotypes.

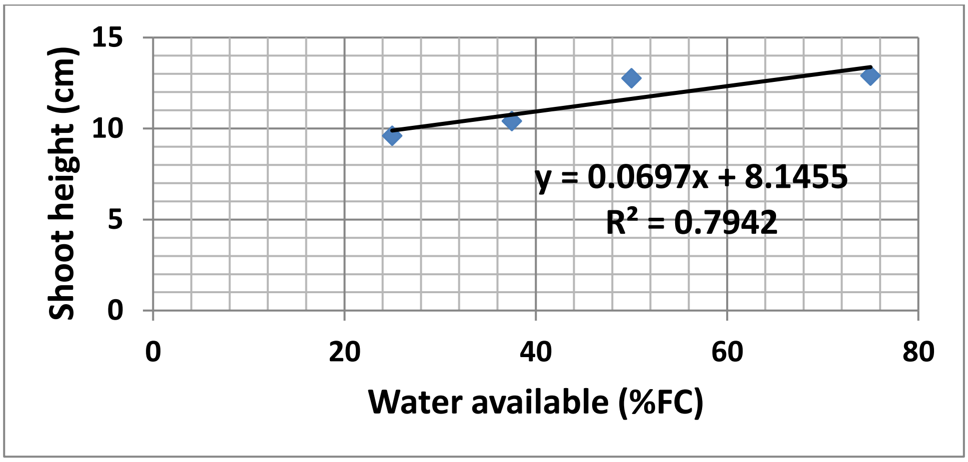

Fig 3: Relationships between shoot height and soil water available of Bambara groundnuts exposed to progressive soil drying at early seedling growing stage. Each point represents the average of 12 values, corresponding to 12 morphotypes.

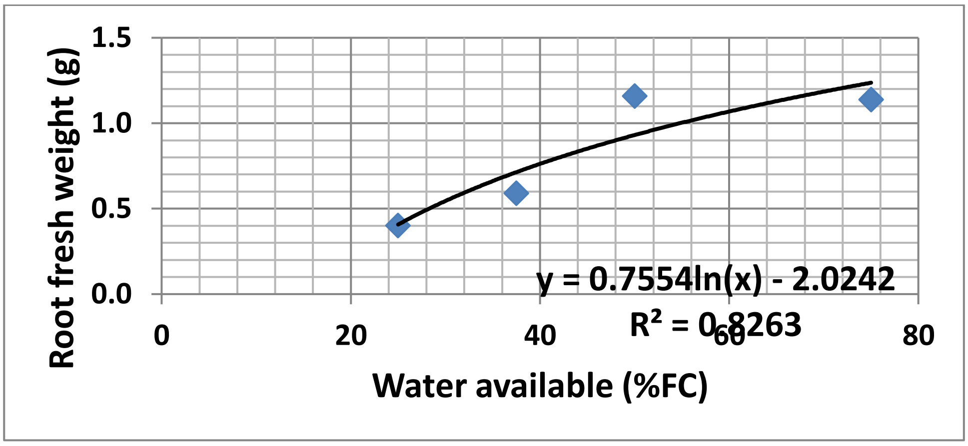

Fig 4: Relationships between root fresh weight and soil water available of Bambara groundnuts exposed to progressive soil drying at early seedling growing stage. Each point represents the average of 12 values, corresponding to 12 morphotypes.

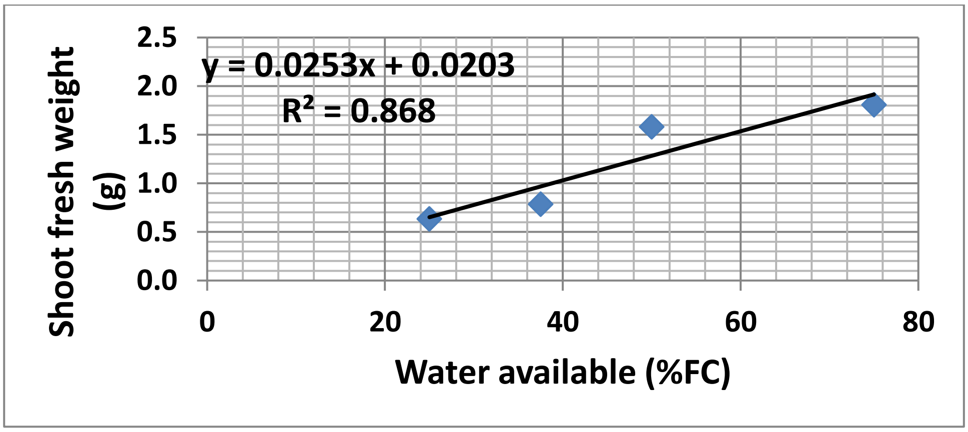

Fig 5: Relationships between shoot fresh weight and soil water available of Bambara groundnuts exposed to progressive soil drying at early seedling growing stage. Each point represents the average of 12 values, corresponding to 12 morphotypes.

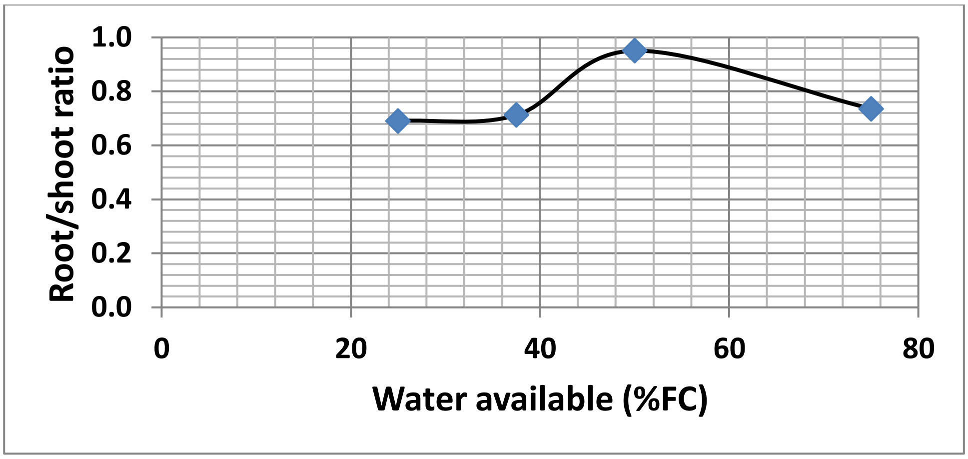

Fig 6: Relationships between root/shoot ratio and soil water available in twelve Morphotypes of Bambara groundnuts exposed to progressive soil drying at early seedling growing stage. Each point represents the average of 12 values, corresponding to 12 morphotypes.

Early stress effect on Bambara groundnut genotypes

Significant differences were observed among morphotypes response to early water stress. NE-04 showed the highest number of leaves (5.0), the highest leaf size (5.3 cm) and the highest shoot and root weight (1.56 and 1.12 g by plant) at 32 days after emergence (DAE). The morphotype NE-12 showed the lowest values of growth index of the entire group. However there was no significant difference in leaf size (Table 3).

Tolerance to drought was measured by the ability of morphotype to minimize the growth reduction due to water deficit. The NE-04 morphotype shows better tolerance with respectively 6.8% and 16.7% decrease in fresh weight and number of leaves (Photo 1). The NE-12 morphotype was most sensitive with respectively 77.0% and 48.6% decrease (Figure 7).

The Drought Susceptibility Index (DSI) varied from 0.20 to 0.94 with an average of 0.42. Only four of twelve genotypes had a DSI value higher than the average, indicating that these individuals stand out from the lot for their relative tolerance to drought (Table 3).

A B C D

Photo1: Genotypic effect of early drought on Vigna subterranea morphotypes; Drought sensitive NE-12 stressed (A) irrigated (B); Drought tolerant NE-04 Stressed (C) irrigated (D)

Table 3: Bambara groundnut genotypic response to water stress: Growth parameters measured after 32 days of growth under water stress condition (W5= 37.5% of soil water holding capacity). Means having the same letters in column are not significantly different at 5% level

Morphotypes	Leaf Number	Plant height (cm)	Leaf size (cm)	Shoot Fresh Weight (g)	Root Fresh Weight (g)	Drought Susceptibility Index(1)
NE-04	5.0a	10.9abcd	5.3	1.56a	1.12a	0.94a
NE-03	4.7ab	12.9ab	3.4	0.74bc	0.27b	0.37bc
NE-05	4.5ab	13.6a	4.3	0.71bc	0.25b	0.69ab
NE-02	4.5ab	10.5bcd	3.8	0.39bc	0.39b	0.21bc
NE-14	4.3ab	8.1d	3.9	0.48bc	0.40b	0.30bc
NE-06	4.3ab	10.4bcd	4.4	0.73bc	0.59b	0.38bc
NE-07	4.3ab	11.7abc	4.6	0.57bc	0.32b	0.37bc
NE-13	4.0ab	9.5cd	4.4	0.70bc	0.45b	0.46bc
NE-09	4.0ab	8.6d	3.4	0.52bc	0.47b	0.31bc
NE-11	4.0ab	8.9cd	4.3	0.97b	0.41b	0.54bc
NE-10	4.0ab	11.7abc	3.4	0.54bc	0.24b	0.33bc
NE-12	3.3b	8.1d	3.2	0.36c	0.30b	0.20c
Average	**4.2**	**10.4**	**4.0**	**0.67**	**0.42**	**0.42**
SD	**0.7**	**2.1**	**0.9**	**0.34**	**0.25**	**0.26**

(1) The drought-susceptibility index calculated according to Fernandez [19] and relative to shoot fresh weight on 32 Days after Emergence

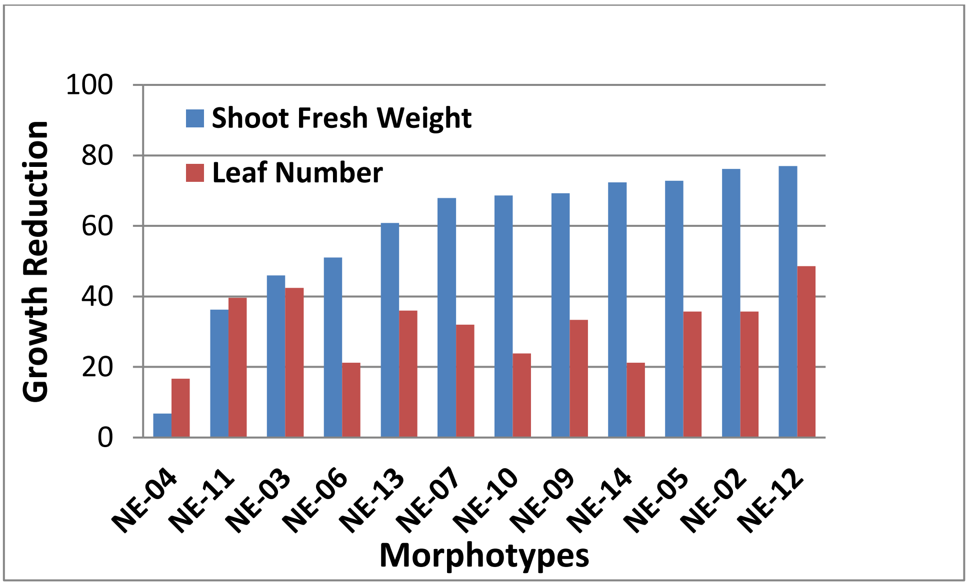

Fig 7: Genotypic effect of early drought on Vigna subterranea morphotypes from Niger

DISCUSSION

The weight of 100 seeds corresponds to what was obtained on morphotypes of Cameroon by Niang *et al.;* [20] and Kouninki *et al.;* [21], but higher than that reported by Dje *et al.;* [22]. There is an interesting variability in the 100 seeds weight and seed color. Several previous studies have focused on the relationships between seed traits and performance of morphotypes. The germination percentage observed is relatively low, below 70%. It will certainly be lower in the natural conditions of BGN culture, due to the erratic rainfall pattern in the Sahelian regions. The low yields are associated with poor germination percentage which leads to poor crop establishment. The best germination was obtained with the NE-07 and NE-09 that are of uniform colors, respectively cream and brown. This result is in accordance with Sinefu *et al.;* [23] working on landrace from Kwa Zulu Natal, South Africa, they found that Brown seeds had the highest germination across treatments, followed by red colored. Colored seeds had higher levels of phenolics which may have led to their enhanced performance during seedling emergence as well as improved drought tolerance and seedling water use efficiency [24]. Thus, seed color may be used as a selection criterion for identifying Bambara landraces with potential for water stress tolerance. Brown color should be a character to be considered by breeders in drought tolerance programs for BGN, for semi-arid regions. Our Results confirmed that seedling growth is significantly affected by water stress, in all growth parameters. The effect of stress began when soil water falls below 37.5% (W4). The W2 water regime (75% FC) was the most suitable water conditions for Bambara groundnut growth, with highest significant values of all growth parameters. The relationship between plant growth and water availability is adjusted by a linear function with very good correlation coefficients. However, the root growth seems better fit on a logarithmic function. The growth of the root system is less active when water availability is sufficient.

The NE-04 morphotype shows better tolerance, with minimal declining growth under water stress (-6.8%), in opposite of NE-12 where growth falls by 77%. The different responses of morphotypes to drought stress may reflect their adaptation to local climate on the collection site. Some previous works [25] reported that local varieties collected in humid regions were more vulnerable to drought than those collected in dry areas. Working on different landraces coming from different climatic zone, Berchie *et al.;* [26] found that Burkina landrace (coming from arid conditions) exhibited the greatest root dry weight and leaf area under drought. Our observations are consistent with these results; indeed, the colorful morphotypes are grown much in the north and central Niger, drier area than the south. Some authors have reported that the Bambara groundnut is more sensitive to drought at pod filling stage [27]. This work shows that early water stress could have a double impact on Bambara Groundnut productivity, by reducing the germination rate (up to 30%) and by lowering the growth rate (up 77%).

The leaf size did not significantly distinguished morphotypes, althoughThe expansion of leaf cells is regulated by the turgor pressure within the cells, and the reduction of turgor potential is directly correlated with reduction of cell extension rate [28].

When soil moisture decrease from 80% to 40%, shoot growth of Vigna subterranea is reduced to a quarter (Figure 5), and crop productivity is affected [29, 30]. These indicate the importance of drought tolerant morphotype for Bambara groudnut productivity in the semi-arid conditions.

CONCLUSION

In confirmation of previous results, this work has highlighted the important existing genotypic variability in the collection of Niger morphotypes of Vigna subterranea. It showed the heavy negative impact of early drought on crop yields of Bambara Groundnut, as a result of low germination rate and low growth in seedling phase. It also shows that the choice of suitable morphotype is an effective solution. The NE-04 morphotype characterized by a seed coat cream striped with brown color offers the best drought tolerance. This promising line could be used as source of tolerant genes for further breeding program.

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