

Article

# Agromorphological Characterization Revealed Three Phenotypic Groups in a Region-Wide Germplasm of Fonio (*Digitaria exilis* (Kippist) Stapf) from West Africa

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Abstract: Fonio is an ancient orphan cereal, cultivated by resource-poor farmers in arid and semi-arid regions of West Africa, who conserved and used the cereal for nutrition and income generation. However, the extent and patterns of phenotypic variation within the fonio germplasm remain scarcely evaluated to inform breeding decisions. In this study, we used alpha lattice design to assess the phenotypic variability of 180 fonio accessions using 20 agro morphological descriptors, including both qualitative and quantitative traits at Bengou research station in 2018 and 2019. Descriptive statistics, combined analysis and multivariate analyses, including principal components analysis (PCA), hierarchical clustering, and multiple factor analysis (MFA) were performed. As results, we found significant differences among fonio accessions and among agro ecological zones of provenance for most of the quantitative traits. Furthermore, highly significant positive correlations were found between grain yield and other yield-related attributes such as harvest index and thousand seeds weight, whereas significant negative correlations were found between grain yield and flowering and maturity times. Clusters analysis revealed three phenotypic groups. Group 1 was characterized by early maturing fonio accessions with higher grain yield. Groups 2 and 3 were characterized by late maturing accessions with intermediate to moderate grain yield. The accessions from Group 1 are candidate for yield improvement and development of fonio lines with enhanced lodging resistance. Accessions from Group 2 and 3 can be improved for yield through marker-assisted selection of best thousand seed weight. This study highlights how traits are correlated within the major phenotypic groups of fonio in West Africa, and we discussed how these groups could be further exploited for



improving traits of economic importance. Furthermore, this study exhibited agro morphological descriptors that discriminate fonio accessions and provide useful information for parental selection with economically important agronomic traits.

**Keywords:** fonio; fonio millet; white fonio; *Digitaria exilis*; agro morphological descriptors; phenotypic diversity; neglected and underutilized species (NUS); genetic improvement

#### 1. Introduction

Fonio (*Digitaria exilis* (Kippist) Stapf), also referred to as white fonio or fonio millet, is one of the oldest cereal crops domesticated by farmers in West Africa. Its cultivation seems to have started about 7000 years ago [1]. Fonio plays a critical role for food and nutrition security for several millions of people in this region, especially during shortage period due to short time life cycle [2]. Besides, its grain is nutritiously valuable due to the presence of two human-vital amino acids methionine and cysteine, which are absent in other staple food cereals such as wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), maize (*Zea mays* L.), or sorghum (*Sorghum bicolor* (L.) Moench) [3]. Additionally, some antithyroid properties have been reported due to high flavonoid content in the crude fonio grain [4]. The grains are poor in gluten and beneficial for people suffering from coeliac disease and overweight people, pregnant and breast feeding women, as well as diabetic people [5]. Furthermore, an efficient formulation of fonio flour enriched with local food resources for the complementary feeding of children under age two has been reported [6]. Due to its C4 metabolism, the crop resists to drought and is well adapted to local pedo-climatic conditions [7].

In traditional fonio farming systems of West Africa, all cultivated varieties are landraces [8]. Their productivity is limited due to the lack of improved varieties and good agronomic practices, lack of institutional support, lack of knowledge about some biotic and abiotic stresses, and tedious post-harvest activities [9]. The development of improved varieties well adapted to different agro ecological zones and preferred by farmers and consumers is of paramount importance in fonio cultivation systems [10]. This can only be achieved by implementing efficient genetic resources management, conservation and valorization, a prerequisite for any crop improvement. This requires a comprehensive knowledge of uses and of diversity patterns. Plant genetic diversity helps breeders in developing improved varieties with high quality, tolerant or resistant to biotic and abiotic stresses, and well adapted to different climate conditions [11].

Morphological markers based on phenotypic characterization of traits are among methods used for assessing diversity within and between plant populations because they are simple, direct, easy and cheap to characterize germplasm accessions. Besides, they are directly related to farmers' perception. Even though these type of markers may present a low level of polymorphism, heritability, and expression and are often susceptible to phenotypic plasticity [12], they were used by many authors to study agro morphological diversity in other small grain cereals [13–16]. Various studies reported the existence of a wide range of diversity in fonio germplasm accessions [17–21] for quantitative traits (plant height, number of leaves per plant, internode length, days to 50% flowering, days to 50% maturity, leaf width, panicle leaf length, stem girth, raceme length, dry biomass yield and grain yield, seeds size, thousand seed weight) and for qualitative traits (collar color, green color of foliar limb, anthocyanin coloration and distribution in different aerial organs, type of panicle and panicle exertion, and grain color). However, previous works on fonio agromorphological characterization limited their collection area to only one or two countries, or used few accessions, overlooking the wider distribution range of the crop. Hence, this limits breeders' chance to get genes of interest, particularly in an orphan crop with a narrow knowledge of the genetic base.

So far, and for the last two decades, the following questions remain unresolved: (a) how is the phenotypic variation distributed among West African fonio accessions? (b) Which agro morphological

traits contribute to distinctiveness in fonio germplasm? (c) How are they related? (d) How many phenotypic groups can be identified? The objectives of this study are to assess phenotypic variability among fonio accessions using agro morphological descriptors, to investigate association patterns among quantitative traits and to identify useful phenotypic groups for future improvement. We hypothesize that exploring a large set of fonio accessions collected from various origins and adaptation areas will enrich the existing germplasm. This will equally help breeders in identifying useful landraces with key agronomic traits for future crop improvement.

# 2. Materials and Methods

# 2.1. Plant Material

A total of 180 fonio accessions were used in this study. They were collected from five Western African countries including Benin (50 accessions), Burkina Faso (five accessions), Guinea (33 accessions), Mali (49 accessions), and Niger (43 accessions), maintained and available in the genebank of the Laboratory of Genetics, Biotechnology and Seed Science (GBioS) from University of Abomey-Calavi (Benin) in 2018. A total of 57 accessions were collected from the arid region, 93 from the semi-arid region, and 30 from the sub-humid region (Figure 1). The arid zone includes the Sahel with up to 750 mm of rainfall in a single short season followed by a long dry season. The vegetation is made up of grass, bush and thicket. In the semi-arid region, the annual rainfall varies from 750 mm to 1250 mm on average in one season followed by a dry season; the vegetation is a savanna grassland. The sub humid region is a deciduous forest and woodland savanna with an average annual rainfall varying between 1250 mm to 1500 mm in one season.

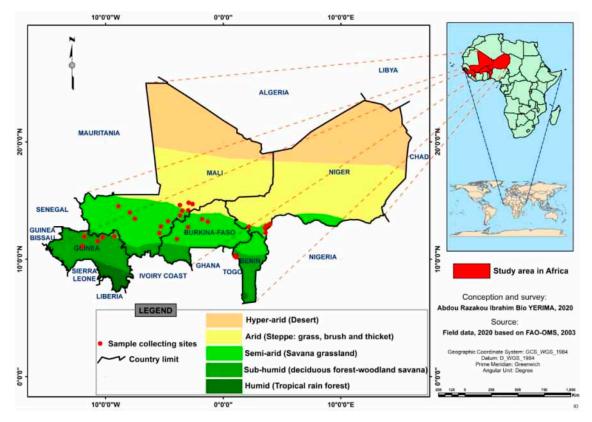


Figure 1. Distribution of collected fonio accessions according to countries and agro ecological zones.

# 2.2. Description of Site

The field trial was carried out at Bengou research station (National Institute of Agronomic Research of Niger) during the rainy seasons 2018 and 2019. This site is in Northern Sudan Savanna

agroecological zone located at 11°58′44.4″ N, 3°32′51.6″ E, at 172 m above sea level and is at 260 km from Niamey and at 17 km form Republic of Benin border. The soil was sandy loam with basic pH medium. Annual rainfall was 1047.62 mm and 1211.2 mm in 2018 and 2019 respectively (Figure 2). The temperature varied between 22.90 °C and 35.20 °C with an average of 29.05 °C. The District of Bengou offers rich and varied vegetation where we can meet almost all the plant species of Niger. The plant population can be subdivided according to the topography and the nature of the soils into three groups: (i) the group of lateritic plateaus is made up of shrub from savannah dominated by species such as *Combretum nigricans* [22], *Combretum micranthum* G.Don [23], *Acacia macrostachya* Rchb. ex DC [24], etc.; (ii) the group of valleys mainly consisted of wooded savanna and classified forests dominated by *Borassus aethiopium*, *Hyphaene thebaica* (L.) Mart [25], *Diospyros mespiliformis* Hochst. ex A.DC, *Parinari macrophylla* (Sabine) Prance ex F.White [26], etc.; and (iii) the group of the old fixed dunes.

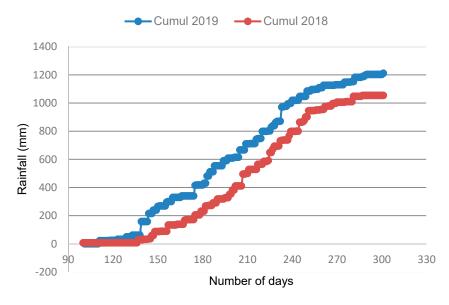


Figure 2. Cumulative rainfall at Bengou research station in 2018–2019.

# 2.3. Experimental Design and Management

The experiment design used was alpha lattice  $(18 \times 10)$  with two replicates and each accession had four rows per plot of 1 m length and 0.20 m apart. The plot area was 1 m<sup>2</sup> and distance between blocks and a replicate was 0.5 m and 1 m respectively. The observations were taken from randomly selected ten sampled plants from the four rows of a plot. Prior to planting, land was ploughed, harrowed and fonio seeds were sown (line sowing) at the rate of 2 g per plot on 19 July 2018 and 18 July 2019. Weeds were rouged out manually three weeks after planting and no fertilizer was applied. Weeding was subsequently done in order to keep the trials clean till harvest. No irrigation was applied during the experiment.

#### 2.4. Data Collection

Data were collected on twenty traits including five qualitative (vigour at seedling. panicle exertion, panicle type, panicle leaf attitude and grain color) and 15 quantitative (plant height, number of internodes per plant, number of leaves per plant, leaf length, leaf width, culm diameter, days to 50% maturity, raceme length, number of racemes per panicle, number of grain par raceme, panicle weight, shoot dry matter yield, grain yield. harvest index and thousand seeds weight) (Table 1). These data were recorded following the Bioversity International rice descriptors list [27].

	Qualitative	
Descriptors	Code	Description
Vigour at seedling	1 = good establishment 2 = moderate establishment 3 = poor establishment	Recorded three weeks after planting
Panicle exertion	1 = well exerted 3 = moderately exerted 5 = lightly exerted 7 = partially exerted	Recorded near maturity
Panicle type	1 = compact 3 = intermediate 5 = open	Recorded near maturity
Panicle leaf attitude	1 = erect 3 = intermediate 5 = horizontal 7 = descending	Recorded after heading
Phenotypic grain colour	1 = brown 2 = greyed orange	Recorded after harvesting
	Quantitative	
Descriptors	Code	Description
Plant height (cm)	PHT	Measured from the soil level to the tip or longest panicle
Number internode	NIN	Mean number of internodes on the mair stem from 10 plants
Internode length (cm)	INL	Measured from the 2nd internode to last internode below the panicle
Number of leaves/plant	NFP	Mean number of leaves from 10 plants
Culm diameter (mm)	CD	Measured at the mid portion of the culm.
Days to 50% flowering (days)	FLO	Date on which 50% of the plants are flowering
Days to 50% maturity (days)	MAT	Date on which 50% of the plants are maturing
Number of racemes/panicle	NRP	Mean number of raceme from 10 panicles
Raceme length (cm)	RLT	Length of main axis measured from the raceme base to the tip
Number of grains/raceme	NGR	Mean number of grain from 10 racemes
Panicle length (cm)	PLT	Measured from panicle leaf insertion to the tip of longest raceme
Dry biomass yield (kg.ha <sup>-1</sup> )	DMY	Ratio of shoot dry weight (kg) to the plot area (ha)
Grain yield (kg.ha <sup>-1</sup> )	GRY	Ratio of grain weight (kg) to the plot area (ha)
Harvest index (%)	HI	Ration of grain yield to total dry biomass × 100
Thousand seeds weight (g)	TSW	Counting and weighting of 1000 seeds

 Table 1. Qualitative and quantitative descriptors for agro morphological characterization of fonio accessions.

#### 2.5. Data Analysis

Descriptive statistics were used to depict variations that emerge from qualitative and quantitative traits. When conditions are met, analysis of variance was performed for quantitative traits in order to firstly verify differences among accessions from various provenances and thereafter from agro ecological zones. Pearson's correlation analysis was carried out to investigate association patterns among quantitative traits. In order to describe the variation of the set of variables together principal components analysis (PCA) [28] was carried out using packages "FactoMineR" [29] and Factoextra [30] in R software version 3.5.1 [31]. Moreover, hierarchical clustering on principal component (HCPC) [32] analysis and linear discriminant analysis (LDA) [33] were used to define hierarchical typologies of fonio accessions and to describe phenotypic groups obtained. Multiple factor analysis (MFA) [34] was used for synthetic comparison of the clusters or phenotypic groups obtained.

# 3. Results

#### 3.1. Variation of Qualitative Traits among Fonio Accessions

Proportion distributions of the qualitative traits (Table 2) revealed that three weeks after planting 30% of accession showed good seedling vigor, while 63% and 7% showed moderate and poor vigor, respectively. Three types of panicle were observed among accessions: compact type (50%), intermediate type (48%), and open type (2%). Panicle leaf attitude was erect (48%), intermediate (45%), or horizontal (7%). Moreover, three types of panicle exertion were noticed with 65% of accessions showing well exerted panicle, 27% moderately exerted, and 8% lightly exerted. Majority of fonio accessions' seed had 84% of brown color, while 16% had greyed orange color (Figure 3a,b).

Descriptors	Modalities	Number of Accessions	Percentage (%)	
	Good	54	30	
Vigour at seedling	Moderate	114	63	
	Poor	12	7	
	Erect	86	48	
Panicle leaf attitude	Intermediate	82	45	
	Horizontal	12	7	
	Compact	90	50	
Panicle type	Intermediate	86	48	
	Open	4	2	
	Well exerted	117	65	
Panicle exertion	Moderately exerted	48	27	
	Lightly exerted	15	8	
Phonotunic grain color	Brown	152	84	
Phenotypic grain color	Greyed orange	28	16	

Table 2. Qualitative parar	neters variations amo	ong fonio accessions.	
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Figure 3. Grain color in fonio accessions, (a): Greyed orange, (b): Brown.

#### 3.2. Fonio Performance Analysis Based on Country of Provenance

Table 3 presents the mean values of quantitative traits of fonio from the different countries. The accessions from Guinea produced significantly taller plant (81 ± 4.92 cm), higher number of internode (11 ± 2.04), and higher internode length (24.24 ± 11.6 cm). This was followed by accessions from Benin with 72 ± 4.62 cm plant height and 10 ± 1.76 internodes. Similar trend was observed for raceme length trait distribution. Accessions with longer racemes were frequent in Guinea (5.52 ± 0.59 cm) and Benin (5.10 ± 1.70 cm) whereas accessions with lower raceme were from Niger and Mali with 4.50 ± 0.60 cm and 4.41 ± 0.93 cm length respectively. However, shorter plants with reduced number of internodes were more predominant among accessions from Niger and Mali with respectively 70 ± 4.98 cm plant height and 7 ± 0.62 internodes, and 68 ± 6.27 cm plant height and 6 ± 0.78 internodes. Accessions from Burkina exhibited medium raceme length (4.80 ± 1.16 cm).

Flowering times (50% of plants flowering) in Guinean fonio accessions was  $85 \pm 7.75$  days, and maturity happens after  $105 \pm 4.57$  days. Flowering time in Malian accession is shorter with  $69 \pm 1.06$  days only. The accessions from Benin exhibited higher number of grain per raceme ( $105 \pm 12.03$  grains) and higher dry biomass yield ( $5095 \pm 145.3$  kg.ha<sup>-1</sup>) followed by Guinea with  $94 \pm 11.9$  grains per raceme and  $5075 \pm 96.47$  kg.ha<sup>-1</sup> of dry biomass yield, respectively. Accessions of Burkina Faso had  $86 \pm 22.3$  grains per raceme and  $4063 \pm$  kg.ha<sup>-1</sup> of dry biomass. Accessions from Niger and Mali exhibited lower values with respectively  $79 \pm 9.18$  and  $76 \pm 11.18$  grains per raceme, and  $4536 \pm 125.8$  kg.ha<sup>-1</sup> and  $3398 \pm 98.27$  kg.ha<sup>-1</sup> for dry biomass yield. In contrast, accessions from Mali and Niger had higher number of leaves per plant with respectively  $212 \pm 70.02$  leaves and  $186 \pm 64.9$  leaves. In addition, accessions from Niger produced higher grain yield ( $1936 \pm 62.25$  kg.ha<sup>-1</sup>) compared to those from Mali ( $1910 \pm 68.75$  kg.ha<sup>-1</sup>), Burkina ( $1063 \pm 70.53$  kg.ha<sup>-1</sup>), Benin ( $1057 \pm 45.99$  kg.ha<sup>-1</sup>), and Guinea ( $924 \pm 45.15$  kg.ha<sup>-1</sup>). Higher harvest index and larger seed size were observed in accessions from Mali and Niger (Table 3).

				Countries				
Quantitative Traits	Benin	Burkina Faso	Guinea	Mali	Niger	Mean	SD	Pr (>F)
Plant height (cm)	$72 \pm 4.62b$	71 ± 12.29bc	81 ± 4.92a	68 ± 6.27c	70 ± 4.98bc	72	5.03	***
Number of internodes	$10 \pm 0.85$ ab	$10 \pm 1.76b$	$11 \pm 2.04a$	$6 \pm 0.78c$	$7 \pm 0.62c$	9	2.17	***
Internode length (cm)	$21.42 \pm 6.1$ ab	22.62 ± 9.6ab	$24.24 \pm 11.6a$	$19.44 \pm 9.3b$	$18.44 \pm 3.9b$	20.73	2.34	**
Number of leaves/plant	$92 \pm 10.8c$	$81 \pm 10.7c$	79 ± 8.1c	$212 \pm 70.2a$	$186 \pm 64.9b$	144	63.85	***
Culm diameter (mm)	$1.48 \pm 0.55a$	$1.40 \pm 0.13$ ab	$1.36 \pm 0.20b$	$1.21 \pm 0.11c$	$1.29 \pm 0.09b$	1.33	0.10	**
Days to flowering	$81 \pm 3.24b$	$80 \pm 2.30b$	$85 \pm 7.95a$	69 ± 1.06d	$74 \pm 2.20c$	77	6.30	***
Days to 50% Maturity	$105 \pm 3.75a$	$98 \pm 3.87b$	$105 \pm 4.57a$	$85 \pm 5.07c$	$84 \pm 1.94c$	95	10.36	***
Number of racemes/panicle	$4 \pm 0.21a$	$4 \pm 0.11a$	$3 \pm 0.14b$	$3 \pm 0.32b$	$3 \pm 0.25b$	3	0.55	***
Raceme length (cm)	$5.10 \pm 1.70b$	$4.80 \pm 1.16 bc$	$5.52 \pm 0.59a$	$4.41 \pm 0.93c$	$4.50 \pm 0.60c$	4.82	0.46	***
Number of grains/raceme	$105 \pm 12.03a$	86 ± 22.3bc	$94 \pm 11.9b$	$76 \pm 11.18c$	$79 \pm 9.18c$	88	11.77	***
Panicle length (cm)	$15.1 \pm 2.56c$	$15.9 \pm 1.73 bc$	$15.8 \pm 1.07c$	$17.2 \pm 3.2ab$	$17.4 \pm 1.40a$	16.4	0.98	**
Dry biomass yield (kg.ha $^{-1}$ )	$5095 \pm 145.3a$	$4603 \pm 159.3$ ab	$5075 \pm 96.47a$	3398 ± 98.27c	$4536 \pm 125.8b$	4490	689.64	***
Grain yield (kg.ha $^{-1}$ )	$1057 \pm 45.99b$	$1063 \pm 70.53b$	$924 \pm 45.15b$	$1910 \pm 68.75a$	$1936 \pm 62.25a$	1474	500.69	***
Harvest index (%)	$26 \pm 4.90c$	$27 \pm 8.98c$	$25 \pm 6.23c$	$59 \pm 9.48a$	$54 \pm 7.18b$	41	16.81	***
Thousand seed weight (g)	$0.59 \pm 0.05d$	$0.63 \pm 0.10c$	$0.68 \pm 0.04b$	$0.82 \pm 0.04a$	$0.69 \pm 0.03b$	0.70	0.09	***

 Table 3. Mean performances of fonio accessions in different countries.

SD = standard deviation. Means followed by the same letter are not significantly different at p = 0.05, \*\*\* = significant at 0.001, \*\* = significant at 0.01.

### 3.3. Fonio Performance Analysis Based on Ecological Zones

Fonio accessions from arid zone produced significantly higher grain yield (2937 ± 327.81 kg.ha<sup>-1</sup>), higher harvest index (60%), and higher thousand seed weight (0.53 ± 0.07 g) (Figure 4m–o). Accessions from sub humid zones revealed lower value with 1364 ± 246.58 kg.ha<sup>-1</sup> grain yield, 30% harvest index, and 0.42 ± 0.05 g thousand seed weight. Furthermore, 50% flowering and 50% maturity times were shorter in accessions from arid zone (72 ± 2.95 days and 93 ± 4.03 days, respectively), whereas accessions from sub humid areas exhibited longer 50% flowering (86 ± 4.55 days) and 50% maturity (108 ± 5.19 days) times (Figure 4f,g). Accessions from the semi-arid zone showed relatively an intermediate 50% flowering and 50% maturity times. Highest number of internode (8 ± 1.19) and the higher internode length (25.62 ± 5.56 cm) were observed in accessions from the sub humid zone. A similar trend was observed for raceme length (9.37 ± 3.97 cm), the number of grain per raceme (88 ± 13.60 grains), panicle length (18.3 ± 1.16 cm), and dry biomass yield (5818 ± 543.87 kg.ha<sup>-1</sup>). However, accessions with wider culm diameter (1.32 ± 0.26 mm) were abundant in the semi-arid zone compared to those from sub humid and arid zones (Figure 4e).

## 3.4. Relationship among Quantitative Traits

We observed highly positive correlations between grain yield (GRY) and harvest index (HI) (r = 0.89), between maturity time (MAT) and flowering time (FLO) (r = 0.80), and between maturity (MAT) and raceme length (RLT) (r = 0.81). Likewise, strong significant positive correlations were observed between number of internode (NIN) and maturity (MAT) (r = 0.77) and between grain yield (GRY) and number of leaves per plant (NLP) (r = 0.68), and thousand seeds weight (TSW) (r = 0.60). However, negative correlations (highly significant and moderately significant) were observed between grain yield (GRY) and flowering time (FLO) (r = -0.79), maturity time (MAT) (r = -0.77), number of internode (NIN) (r = -0.76), and plant height (PHT) (r = -0.35). Moreover, strong significant negative correlations were found between harvest index (HI) and flowering (FLO) (r = -0.84), and maturity (MAT) (r = -0.79) (Table 4).

	Table 4. Pearson's correlation matrix on fifteen	quantitative traits used in f	onio genotypes description.
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					65	1447				NURR	NOR		GRV		-
	PHT	NIN	INL	NLP	CD	MAT	FLO	PLT	RLT	NRP	NGR	DBY	GRY	HI	TSW
PHT	1														
NIN	0.53	1													
INL	0.55	0.77	1												
NLP	-0.38	-0.75 *	-0.70	1											
CD	0.35	0.53	0.51	-0.51	1										
MAT	0.36	0.77 **	0.70	-0.71	0.51	1									
FLO	0.35	0.77	0.64	-0.71	0.56	0.80 **	1								
PLT	0.46	0.25	0.34	-0.19	0.02	0.24	0.05	1							
RLT	0.57	0.74	0.73	-0.64	0.42	0.81 **	0.66	0.49	1						
NRP	-0.02	0.21	0.16	-0.27	0.31	0.27	0.23	-0.21	0.13	1					
NGR	0.43	0.69	0.64	-0.60	0.50	0.64	0.67	0.15	0.61	0.30	1				
DBY	0.48	0.65	0.52	-0.50	0.43	0.51	0.66	0.17	0.53	0.11	0.59	1			
GRY	-0.35 **	-0.76 **	-0.70	0.68 *	-0.42	-0.77 *	-0.79 **	-0.18	-0.69	-0.17	-0.56	-0.43	1		
HI	-0.37	-0.76	-0.74	0.69	-0.48	-0.79 **	-0.84 **	-0.21	-0.69	-0.16	-0.61	-0.62	0.89 **	1	
TSW	-0.24	-0.63	-0.45	0.61	-0.53	-0.64	-0.79	0.08	-0.49	-0.34	-0.62	-0.60	0.60 **	0.66	1

PHT = plant height. NIN = number of internodes, INL = internode length, NFP = number of leaves per plant, CD = culm diameter, FLO = 50% flowering, MAT = 50% maturity, NRP = number of racemes per panicle, PLT = panicle length, RLT = raceme length, NGR = number of grain raceme, DBY = dry biomass yield, GRY = grain yield, HI = harvest index, TSW = thousand seeds weight, \* = significance at 0.05, \*\* = significance at 0.01.

90

70

60

400

300

Arid zone

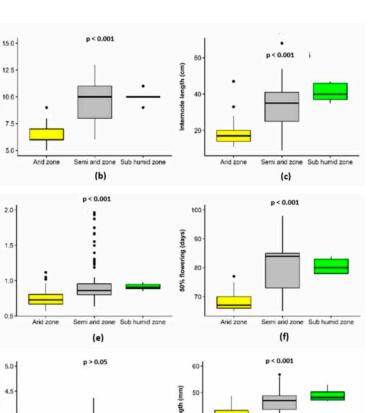
Plant height (cm) 80 p < 0.001

Semi arid zone Sub humid zone

(a)

p < 0.001

Number of inter



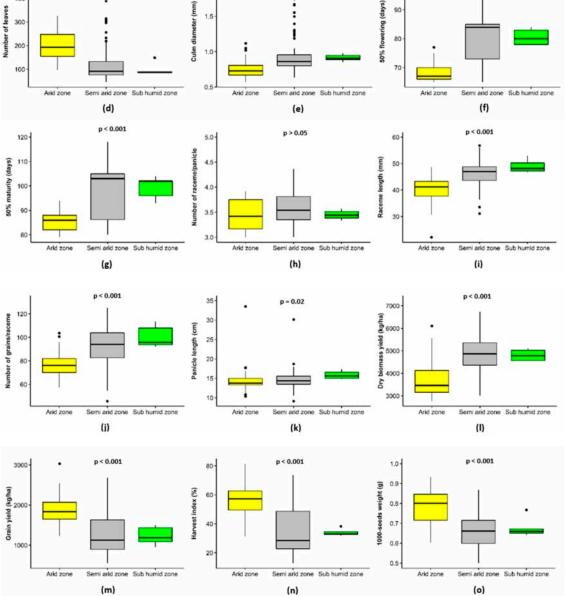
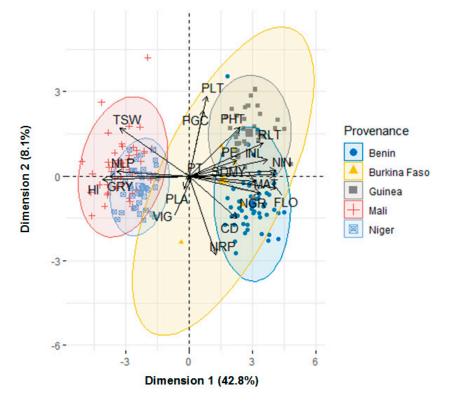


Figure 4. Boxplot showing fonio accessions performance based on ecological zones. Variation of: (a) plant height, (b) number of internodes, (c) internodes length, (d) number of leaves, (e) culm diameter, (f) 50% flowering, (g) 50% maturity, (h) number of racemes per panicle, (i) raceme length, (j) number of grains per raceme, (k) panicle length, (l) dry biomass yield, (m) grain yield, (n) harvest index, (o) 1000 seeds weight.

#### 3.5. Clustering of Accessions Based on Country of Provenance

The results of PCA showed that the first two dimensions accounted for 50.90% of the total variation among fonio accessions. The first dimension (Dimension 1) was highly correlated with flowering time, number of internodes, maturity time, internode length, raceme length, number of grain per raceme, dry biomass yield, thousand seed weight, number of leaves per plant, grain yield and harvest index while the second dimension (Dimension 2) was moderately correlated to number of raceme per panicle, panicle length, phenotypic grain color, culm diameter, and plant height (Figure 5). Furthermore, the results of principal component analysis (PCA) showed that fonio accessions from Benin, Burkina Faso, and Guinea were characterized by late flowering and maturing accessions, with taller plants, long raceme length and higher number of grains per raceme compared the group of accessions from Mali and Niger that were characterized by early flowering and early maturing accessions, with higher grain yield, higher thousand seeds weight and higher number of leaves of per plant. Moreover, the PCA results showed that Dim1 differentiates two groups of fonio accessions: one group composed essentially of accessions from Mali and Niger, while the second group was composed of accessions from Benin, Burkina, and Guinea. Likewise, Dimension 2 differentiates two groups of accessions, the first group was composed of accessions from Guinea, Benin, and Burkina Faso, while the second group was again a mixed of accessions from these three countries (Figure 5).

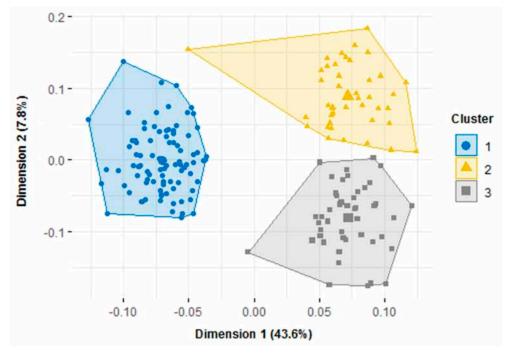


**Figure 5.** Principal components analysis biplot showing variables and individuals on Dimensions 1 and 2.

# 3.6. Partition of Accessions into Phenotypic Groups

The hierarchical clustering on principal components analysis grouped fonio accessions into three different clusters or phenotypic groups (Figure 6). Cluster 1 (50.56% of total accessions), essentially gathered accessions from Mali and Niger, characterized by early flowering and early maturing genotypes (respectively means of  $69 \pm 3.06$  days and  $86 \pm 3.99$  days), and short plants (PHT =  $69 \pm 5.76$  cm), and high number of leaves per plant (NLP =  $202 \pm 69.74$  leaves per plant). Accessions of Cluster 1 produced higher average grain yield (GRY =  $1280 \pm 660.10$  kg.ha<sup>-1</sup>), with higher harvest index (HI = 50%) and the higher thousand seeds weight (TSW =  $0.76 \pm 0.07$  g). Cluster 1

was predominantly composed of brown color accessions with well exerted panicle. Even though, accessions from this phenotypic group showed high performance in terms of yield, they produced less vigorous plant (CD =  $0.77 \pm 0.11$  mm), and recorded lower values of number of grains per raceme (NGR =  $77 \pm 10.39$  grains), with intermediate raceme length ( $8 \pm 0.79$  cm) and panicle length  $(14 \pm 1.63 \text{ cm})$  (Table 5). Cluster 2 (23.89% of total accessions) and Cluster 3 (25.55% of total accessions) were composed of late flowering (FLO =  $84 \pm 5.21$  days for Cluster 2 and  $86 \pm 3.38$  days for Cluster 3) and late maturing accessions (MAT =  $104 \pm 5.50$  days for Cluster 2 and  $105 \pm 3.38$  days for Cluster 3). These phenotypic groups (Cluster 2 and 3) were predominantly composed of accessions with long to intermediate plant height (PHT =  $79 \pm 5.42$  cm for Cluster 2 and PHT =  $72 \pm 5.33$  cm for Cluster 3), with more vigorous plants (CD =  $1.14 \pm 0.38$  mm for Cluster 3 and CD =  $0.87 \pm 0.09$  mm for Cluster 2), and with long racemes (RLT =  $9.90 \pm 0.61$  cm for Cluster 2 and RLT =  $9.40 \pm 0.58$  cm for Cluster 3) and long panicles (PLT =  $17 \pm 3.54$  cm for Cluster 2, PLT =  $14 \pm 1.05$  cm for Cluster 3). Accessions of these two Cluster recorded intermediate number of leaves per plant and intermediate grain yield  $(GRY = 2048 \pm 450.40 \text{ kg.ha}^{-1} \text{ for Cluster 3 and } GRY = 2019 \pm 550.80 \text{ kg.ha}^{-1} \text{ for Cluster 2})$ . However, accessions from Cluster 3 recorded higher number of grains per raceme (NGR =  $104 \pm 14.27$  grains) than accessions from Cluster 2 (NGR =  $96 \pm 13.22$  grains). The same trend was observed for number of racemes per panicle (NRP =  $4 \pm 0.21$  racemes for Cluster 3 and NRP =  $3 \pm 0.23$  racemes for Cluster 2).



**Figure 6.** Hierarchical clustering on principal components analysis (HCPC) showing number of clusters and individuals within clusters in fonio accessions.

Table 5. Description of phenotypic groups in fonio accessions.	

Variables	Cluster 1, N = 91 ML = 48 and NG = 43	Cluster 2, N = 43 BN = 6, BF = 2, GN = 33, ML = 2	Cluster 3, N = 46 BN = 43 and BF = 3	Pr (>F)
	$Mean \pm SD$	$Mean \pm SD$	$Mean \pm SD$	
Plant height (cm)	$69 \pm 5.76$	$79 \pm 5.42$	72 ± 5.33	***
Number of internodes/plant	$7 \pm 0.77$	$11 \pm 1.20$	$10 \pm 1.02$	***
Internode length (cm)	$19 \pm 7.69$	$39 \pm 6.16$	$37 \pm 6.68$	**
Number of leaves/plant	$202 \pm 69.74$	$90 \pm 23.84$	$83 \pm 17.75$	***
Culm diameter (mm)	$0.77 \pm 0.11$	$0.87 \pm 0.09$	$1.14 \pm 0.38$	***
Flowering (days)	$69 \pm 3.06$	$84 \pm 5.21$	$86 \pm 3.38$	***
Maturity (days)	$86 \pm 3.99$	$104 \pm 5.50$	$105 \pm 3.38$	**
Panicle exertion	$1 \pm 0.68$	$3 \pm 1.42$	$3 \pm 1.48$	***
Raceme length (cm)	$8.1 \pm 0.79$	$9.90 \pm 0.61$	$9.4 \pm 0.58$	***
Panicle length (cm)	$14 \pm 1.63$	$17 \pm 3.54$	$14 \pm 1.05$	***
Number of racemes/panicle	$3 \pm 0.29$	$3 \pm 0.23$	$4 \pm 0.21$	***
Number of grains/raceme	$77 \pm 10.39$	$96 \pm 13.22$	$104 \pm 14.27$	**
Grain yield (kg/ha)	$3840 \pm 660.10$	$2019 \pm 550.80$	$2048 \pm 450.40$	**
Harvest index (%)	$57 \pm 8.78$	$26 \pm 7.68$	$26 \pm 5.43$	**
Thousand seeds Weight (g)	$0.76\pm0.07$	$0.68\pm0.06$	$0.59\pm0.04$	ns

SD = standard deviation, BN = Benin, BF = Burkina Faso, GN = Guinea, ML = Mali, NG = Niger. \*\*\* = significance at 0.001, \*\* = significance at 0.01, ns = not significant.

The HCPC analysis was supported by linear discriminant analysis (LDA) (Figure 7). The LDA results showed that linear discriminators LD1 and LD2 clearly separate the three phenotypic groups without admixture, while LD1 and LD3, LD1 and LD4 are fairly good at separating two to three Clusters. The result of multiple factor analysis (MFA) showed a synthetic comparison of the three phenotypic groups. This method groups variables in different classes of morphology, phenology and yield before making comparison between phenotypic groups. Dimension 1 clearly differentiates Cluster 1 from Clusters 2 and 3, while Dimension 2 differentiates Cluster 1 from Cluster 2.

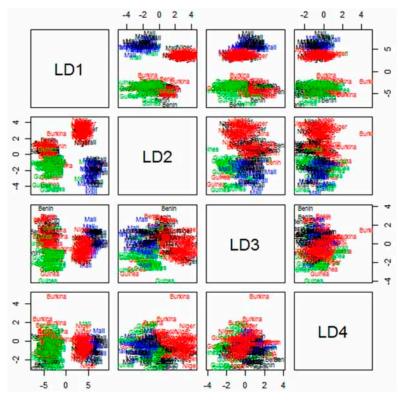


Figure 7. Linear discriminant analysis (LDA) in fonio.

#### 4. Discussion

# 4.1. Qualitative Traits Variation in Fonio

Qualitative traits analysis revealed that most of the fonio accessions had well exerted panicle and brown grain color. Panicle exertion is important in cereal research though this was rarely documented in fonio for which a persistent interest for higher yielding cultivars is nowadays expected. Panicle exertion was reported as a crucial morphological marker that could be used for identification of hybrid plants derived from crosses between cultivated rice and African wild rice (Oryza longistaminata A. Chev. & Roehrich) [35]. The effect of genetic and abiotic factors on the panicle exertion in fonio is a topic of interest for future investigations. Seed color is one of the most important traits that guide breeders, farmers, processors, as well as consumers for cultivar preference [36,37]. In fonio, seed color was often associated with other traits including crop cycle, yield, tolerance to biotic and abiotic stresses, harvesting and processing abilities [3], though this needs to be fully documented. The brown seeded accessions, commonly known as white fonio (reference to *D. exilis*), is the most diverse and widely cultivated cultivar in the region [38]. Except in Niger where the majority of fonio accessions had greyed orange color [21], the predominance of brown seeded color in West Africa could be explained by its well adaptability to various pedo-climatic conditions and its association with other desirable agronomic traits. Lule et al. [39] reported that farmer's preferences of grain color (purple black) in finger millet (*Eleusine coracana* (L.) Gaertn.) was associated with adaptability to environment factors, yielding ability, major use of the grain, or cultural role and ease of production in northern Ethiopia. Therefore, future research should investigate the genetic basis of the seed color and its association of functional agronomic traits.

#### 4.2. Performance of Fonio Accessions Based on Provenance

Our findings revealed that grain yield in fonio from various countries and ecological zones were higher than that reported previously [18]. Similar trend was observed for number of internode and internode length which were also higher compared to results obtained by Saidou et al. on Niger accessions only [21]. The mean thousand seeds weight recorded by Nyam [20] was also lower than that obtained in this study. However, higher values for plant height, number of internode, number of raceme par panicle, raceme length, and panicle length were reported by Sekloka et al. [19] in the characterization at the University of Parakou (Rep. of Benin) of twenty fonio accessions collected from Boukoumbe. This is an illustration of the morphological diversity among fonio accessions which could be due to the expression of genes controlling complex traits, or environmental factors including agricultural practices [10,18,40]. The significant differences observed among fonio accessions for most of the quantitative traits could also be attributed to the variability in rainfall patterns because the cumulative rainfall recorded in 2019 (1211.20 mm) was much higher than that of 2018 (1047.62 mm). This suggests that the amount and distribution of rainfall impact the performance of fonio accessions during the two consecutive years of experiment. Dehgahi et al. [41] reported also that grain yield was positively correlated with the average annual rainfall in wheat. That variability of rainfall can trigger the disruption in crop production and food insecurity [42].

Differences observed among accessions of various provenances indicated that the variation of most quantitative traits may be likely due to adaptation to specific environment and sowing date. Differences for flowering and maturity times were conspicuous among accessions. The early maturing accessions were more frequent in relatively hot and dry areas of arid zone from Mali and Niger whereas the late maturing accessions occurred abundantly in semi-arid and sub-humid areas from Benin, Burkina Faso and Guinea. This suggests that fonio accessions from the arid zone might have lost their photoperiod sensitivity in trying to adapt to prolonged dry spells and intensified heat waves compare to those from sub-humid zone which might be photoperiod sensitive with less drought spells and heat waves. Earlier works reported highly significant differences for number of days to flowering as affected by photoperiods within *D. exilis* [43]. In pearl millet (*Pennisetum glaucum* (L.) R. Br.), one of major crops

cultivated in the dryland areas of West Africa, similar results were reported by Haussmann et al. [44] that long-cycle millets showed a stronger sensitivity to photoperiod than shorter-cycle cultivars in West and Central African pearl millet landraces.

In this study, fonio accessions were sown around mid-July with the consequence of exposing them to flower late in long days. Ordinary, farmers from semi-arid and sub humid zones sow fonio earlier in May. This shift in sowing date might have led to the lower grain yield observed in fonio accessions from sub-humid zone compared to those from arid zones. Gueye et al. [45] also reported that the delay in sowing dates reduced grain yield by 50% to 87% in fonio. Our result was in accordance with those of Wolabu and Tadege [46], who reported that regardless of the day length, non-photoperiod sorghum genotypes flower earlier than photoperiod sensitive genotypes. Future research will certainly decipher the effect of specific environment on the yield and yield components through a multiple environmental trial in order to reveal the ability of each ecological zone to contribute to yield accumulation in Fonio. This will clearly highlight the vulnerability and adaptability of fonio along the ecological gradient of west Africa as this was investigated for wheat in Parkistan [47].

## 4.3. Quantitative Traits Association and Partition of Phenotypic Groups in Fonio

Correlation analysis revealed highly significant positive relationships between grain yield and harvest index, thousand seed weight, number of leaves. Traits with high significant positive correlations would contribute to grain yield improvement in fonio. This implies that those traits can be used as proxy for yield in indirect selection and harvest index could be recommended as a selection criterion for increasing yield in fonio. In finger millet, similar results were reported that grain yield was positively associated with harvest index [48]. However, number of internode and plant height negatively impacted grain yield. Likewise, long flowering and maturing times negatively influenced grain yield had highly significant positive correlation with maturity and flowering times in finger millet [50]. In addition, grain yield exhibited moderate negative correlation with plant height indicating that higher grain yield is associated with shorter fonio plants. This is an important feature in tackling lodging problem which is the main cause of yield losses and quality reduction in fonio production in West Africa. So, breeding for shorter plant could enhance lodging resistance in fonio varieties. Similar results were reported in tef (*Eragrotis tef* (Zucc.) Trotter) [51].

Yield, yield components, and phenological traits were the contributors to the variation observed in this study. Clusters analysis and linear discriminant analysis (LDA) showed three clusters of fonio accessions. A previous report indicated four clusters while characterizing 20 fonio accessions only from Benin [19]. Another report revealed two groups in the evaluation of 30 fonio accessions from Nigeria [20]. Our findings cover accessions from five countries (Benin, Burkina Faso, Guinea, Mali, and Togo) and three phytogeographical areas. However, accessions from Nigeria should be added for a broader perspective on the morphological variation in fonio in relation to different agro ecological zones in order to enrich the cultivated germplasm from West Africa. With the clear separation of phenotypic groups, it becomes critical to revisit the level of genetic diversity within fonio germplasm [18] and the association between genetic and phenotypic features for proper improvement of the crop. We found that fonio accessions clustered in phenotypic groups based on their provenance; though accessions from Benin and Mali that were distributed throughout Cluster 1 and Cluster 2. Amplified fragment length polymorphisms (AFLPs) analysis of 122 fonio accessions [18] revealed similar trends where fonio accessions from Benin, Burkina Faso, Guinea, and Togo clustered in identical group based on their origin except accessions from Guinea and Mali. In addition to site specificity, genes flow is a powerful mechanism that contribute to homogenization of variability distribution. For instance, grouping of accessions regardless of boundaries reflect the importance of farmers' seed exchange in fonio seed systems. As informal seed systems, seed exchange is commonly practiced by fonio farmers as reported by previous works [2,10,52].

Group 1 was composed essentially of early flowering and maturing accessions, and recorded higher values for grain yield, harvest index and thousand seeds weight. The accessions of this group are adequate candidates for developing early maturing fonio lines for arid and semi-arid regions where drought stress occurs frequently. Furthermore, this group is composed of fonio accessions with shorter plants that could be used as source for lodging resistance since short stature plants have been reported in cereals to be preferred in varietal development for that trait. Phenotypic Groups 2 and 3 were composed predominantly of late maturing accessions with long to intermediate plant height, with higher values of culm diameter. Moreover, these groups were composed of accessions with long racemes and long panicles. The difference observed in the groups could be likely due to the genetic make-up of each genotype and prevailing environmental factors. However, despite their phenotypical similitude, accessions from Group 3 recorded higher number of grains per raceme and number raceme per panicle than accessions from Group 2 revealing another agronomic trait of importance for the improvement of the crop. Marker assisted index selection of fonio lines with long raceme, and long panicle alongside with high number of grains per raceme and high number of racemes per panicle can be explored as crop a improvement avenue. Moreover, genome-wide association studies (GWAS) are needed to understand the genetic basis of key important agronomic traits in fonio.

## 5. Conclusions

This study assessed phenotypic variability among 180 fonio accessions from five West African countries. It revealed the existence of wide range of agro morphological variability. The results revealed interesting correlations between yield and yield components. Furthermore, clusters analysis revealed three phenotypic groups. Group 1 was composed of early flowering and maturing accessions with higher grain yield, higher harvest index, higher thousand seeds weight, and higher number of leaves per plant. Accessions from Group 2 and 3 were characterized by late flowering and maturing times, with intermediate grain yield, thousand seeds weight, and number of leaves per plant, with taller plants, higher values of number of grains per raceme, and number of racemes per plant. Accessions from Group 1 are candidates for yield improvement and enhancing lodging resistance, whereas accessions from Group 2 and Group 3 are candidate also for yield improvement via indirect selection and good parent for hybrids development. The study exhibited agro morphological descriptors that discriminate fonio accessions and provide also useful information for parental selection with key economically important agronomic traits.

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