Genetic Variability, Heritability and Genetic Advance in Pearl Millet (*Penisetum glaucum* [L.] R. Br.) Genotypes

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Authors' contributions

This work was carried out in collaboration between the two authors. The authors read and approved the final manuscript.

ABSTRACT

Fifteen genotypes of pearl millet (*Pennisetum glaucum* L. R. Br.) were evaluated at Sudan University of Science and Technology, The Demonstration Farm, College of Agricultural Studies, Shambat, during the summer season 2009 and 2010. The present study was conducted to assess the magnitude of genetic variability, heritability in broad sense and genetic advance among fifteen pearl millet genotypes for some growth and grain yield characters. A randomized complete block design with three replications was used at each season. Highly significant differences (P≤ 0.01) were observed for days to 50% flowering and days to maturity in the both seasons, for plant height, leaf area, number of grains /plant, 1000 grain weight and grain yield (t/ha) in the summer season of 2009, for panicle length in the summer season of 2010. Also highly significant differences were observed for genotypes and genotypes × seasons interaction for days to 50% flowering and days to maturity. In general phenotypic coefficients of variation (PCV) estimates were higher than genotypic coefficients of variation (GCV) estimates for all the studied characters in all genotypes displaying the influence of environment effect on the studied characters. The combined results for heritability showed that the high estimates of heritability and genetic advance were scored for days to 50% flowering and days to maturity indicating that these characters were under the control of additive genetic effects. The genotypes ICMV155

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and SADC (long) scored the most minimum days to maturity (68) days whereas, the genotypes ICMW221 and Ugandi scored the highest yield values of 2.20 and 2.05(t/ha), respectively. Such genotypes can be manipulated for further improvement in millet breeding programs at the Sudan.

1. INTRODUCTION

Pearl millet (*Pennisetum glaucum* [L.] R. Br.) is an annual and cross pollinated crop, have chromosome number of 2n =14. Pearl millet is believed to be originated in west Africa, the term millet is brooding applied over 140 species belonging to the Genus *Pennisetum* [1,2]. It is planted as a grain and/or fodder crop across a wide range of environments around the world. It is of a great importance in the arid and semi–arid tropics, where it is the stable food for millions of people. Today millet cover the food needs for more than 500 million people, areas planted primarily with millet are estimated by 15 m ha annually in Africa and 14 m ha in Asia [3]. In Sudan, pearl millet is the preferred as a staple food for the majority of inhabitants in Western Sudan (Kordofan and Darfur states). Among the cereals (grains), it comes second to sorghum in cultivated areas and total production which estimated by 2.1 million hectares and 871 thousand tones, respectively and it equals about 50% of sorghum cultivated areas and total production [4]. This is mainly raised under traditional farming methods, where the rainfall is between 200 – 800 mm [5] and the average yield was 653 kg/ha [6]. Fadlalla [7] and Abuali [8] reported that the main reason leads to low yield of pearl millet is due to lack of hybrid pearl millet varieties and high yielding cultivars suited to Sudan environment. Therefore, need to investigation of wide range of variability among pearl millet genotypes and isolate the suitable genotype(s) that perform well under Sudan environment. Variability for different characters present in germplasm collections is important for a successful pearl millet breeding program. The progress of selection is more important in any crop improvement and this progress is depends on the existence of genetic variability for yield and yield contributing characters and their heritability [9]. Heritability in conjunction with genetic advance has a greater role to play in determining the effectiveness of selection of a character [10]. Therefore, the present study was conducted to assess genetic variability, heritability and genetic advance among fifteen pearl millet genotypes in two consecutive seasons in order to select the appropriate genotype(s) that are suited to Sudan environment.

2. MATERIALS AND METHODS

The genetic material used in this study is consisted of fifteen pearl millet (*Pennisetum americanum* [L.] R.Br.) genotypes from different environments and pedigrees. Out of fifteen, ten were introduced and selected by Agricultural Research Corporation (ARC), Sudan and the other five were collected from different locations at Darfur states, West of Sudan (Table 1). The Field experiments were carried out at two consecutive summer seasons of the years 2009 and 2010 at The Demonstration Farm, College of Agricultural Studies, Sudan University of Science and Technology, Shambat (15º 40′ N, 32º 32′ E).

Table 1. List of pearl millet genotypes used in the study and their pedigree

	Designation	Source and pedigree
1	ICMV 221	Open pollinated variety introduced from ICRISAT by ARC, Sudan.
2	Ashana	Okashana-2 variety, introduced and released under the name of Ashana by ARC, Sudan.
3	ICMV 155	Open pollinated variety introduced from ICRISAT by ARC, Sudan.
4	MCSRC	Elite variety introduced from ICRISAT by ARC, Sudan.
5	SADC (Long)	White grain population introduced from ICRISAT by ARC, Sudan.
6	SADC (Togo)	High grain yield population introduced from ICRISAT by ARC, Sudan.
7	Ugandi	Introduced from Uganda and released by ARC, Sudan.
8	Sudan II	Population developed and improved by recurrent selection at ARC, Sudan.
9	Sudan III	Population developed and improved by recurrent selection at ARC, Sudan.
10	MCNELC	New Elite Composite Variety introduced from ICRISAT by ARC, Sudan.
11	Dembi Millet	Local variety at East Darfur State, Sudan.
12	Dembi Shangal Toby	Local variety at South Darfur State, Sudan.
13	Dembi Elfasher	Local variety at Middle Darfur State, Sudan
14	Dembi Kabkabia	Local variety at West Darfur, Sudan.
15	Dembi Sea	Local variety at North Darfur State, Sudan.

ARC = Agricultural Research Corporation, Sudan.
ICRISAT = International Center Research Institute Semi Arid Tropics, India.

The experiments were laid out in a randomized complete block design (RCBD) with three replications. The experiment site was disc ploughed, disc harrowed and leveled, ridging up was north-south, 70 cm apart. The land was divided into 3 x 4 m plots, each composed of 4 ridges, three meters long, seeds were sown manually along the ridges in holes 20 cm apart. Sowing date was the 6th of July 2009 and 7th of July 2010 for the two summer seasons. Seed rate applied was 2.5 kg /fed., Nitrogen fertilizer (urea 46% N) 80 Kg/F was applied in two equal doses after three and six weeks from sowing date, respectively. Hand weeding was conducted when needs. Irrigation was scheduled at 7- 10 days intervals. Ten randomly plants were selected from the two inner ridges at each plot and were used for data collection at each location on all the traits except days to 50% flowering and days to maturity, viz., namely days to 50% flowering, days to maturity, plant height(cm), number of leaves/plant, number of tillers /plant, panicle length (cm), number of grains/plant, grain yield/plant, 1000-grain weight (gm) and grain yield (t/ha). Days to 50% flowering and days to maturity were computed on plot basis. Analysis of variance was carried out by M.STAT computer software according to the procedures described by Gomez and Gomez [11] for each season individually and for the combined seasons. The estimates of phenotypic (σ^2ph) and genotypic (σ^2g) variances were worked out according to the method suggested by Johnson et al. [12] using mean square values from the individual and combined ANOVA tables as the following formula:

a. For the individual analysis of variance, they were estimated as follows:

$$\sigma^2 g = (M_2 - M_1)/r$$

$$\sigma^2 ph = \sigma^2 g + \sigma^2 e$$

Where:
  r = number of replications.
  $\sigma^2 e$ = error or environmental variance.`
  M_1, M_2 = error and genotype mean squares.

b. For combined analysis of variance, they were estimated as follows

Genotypic variance $(\sigma^2 g) = (M_2 - M_1)/rS$

Phenotypic variance $(\sigma^2 ph) = \sigma^2 g + \sigma^2 gS + \sigma^2 e$
Where:
  g = number of genotypes
  S and r = number of seasons and replications, respectively.
  $\sigma^2 e$ = error or environmental variance .
  M_1 = expected mean squares of pooled error
  M_2 = expected mean squares of genotypes x seasons interaction.

Phenotypic (PCV) and genotypic (GCV) coefficients of variation (individually and combined) were calculated based on the method advocated by [13] as the following formula:

Phenotypic coefficient of variation (PCV) = $\dfrac{\sqrt{\sigma^2 Ph}}{\text{Grand mean}} \times 100$

Genotypic coefficient of variation (GCV) = $\dfrac{\sqrt{\sigma^2 g}}{\text{Grand mean}} \times 100\%$

Heritability percentage in broad sense $h^2(bs)$ and Genetic advance (GA) were estimated according to the method suggested by [12]   Johnson et al. (1955) as the following formulas:

1a. $h^2(bs)$ from individual analysis of variance:
$$h^2(bs) = \sigma^2 g / \sigma^2 ph$$
Where:
  $\sigma^2 g$, $\sigma^2 ph$ = genotypic and phenotypic variances.
1b. $h^2(bs)$ from combined analysis of variance: It was calculated as a ratio of the genotypic variance to the phenotypic variance according to the formula :

$$h^2(bs) = \sigma^2 g / \left[\sigma^2 g + \frac{\sigma^2 gL}{r} + \frac{\sigma^2 e}{rS}\right]$$

2a. (GA) from individual analysis of variance: $= K \dfrac{\sigma^2 g}{\sqrt{\sigma^2 Ph}}$

2b. (GA) from combined analysis of variance = $K \dfrac{\sigma^2 g}{\sqrt{\left[\sigma^2 g + \frac{\sigma^2 gL}{r} + \frac{\sigma^2 e}{rS}\right]}}$

Where:

$\sigma^2 g$ = is the estimated genetic variance
$\sigma^2 gS$ = the variance due to genotypes x seasons interaction .
$\sigma^2 e$ = is the pooled error variance
S and r = are a number of seasons and replications, respectively.

K= selection differential and it was 2.06 as defined by [14] at selection intensity of 5%.

3. RESULTS AND DISCUSSION

The results of the individual and the combined analysis of variance are presented in Table 2. The individual analysis of variance showed wide range of variation, Highly significant differences ($P \leq 0.01$) were observed for days to 50%flowering and days to maturity in the two seasons, for plant height, number of grains /plant, 1000 grain weight and grain yield (t/ha) in the first season(2009) and for panicle length in the second season (2010). The combined analysis of variance showed highly significant differences for genotypes and genotype × seasons interaction for most of the characters. This variation could be attributed to genetic and environmental effects as well as their interactions. Substantial variations in pearl millet have been also reported in previous studies by many investigators [15,16,17,18]. The measurement, evaluation and existence of variability are essential steps in drawing meaningful variability and better conclusions from a given set of phenotypic observations [19,20]. The means of the growth and yield characters obtained by the fifteen millet genotypes are presented in Table, 3. The genotypes ICMV155 and SADC (long) scored the most minimum days to maturity (68) days and a moderate values of yield per ton/ha comparing with other millet genotypes used in the study, indicated the availability of using them at drought areas, in millet hybrid industry and/or as a selected millet genotypes characterized with early maturity and promising for high yield at Sudan. The results showed that the genotypes ICMW221 and Ugandi scored the highest mean values for yield of 2.20 and 2.05 (t/ha), respectively. Such genotypes could be recommended for general cultivation under field conditions of Sudan to help farmers to compensate their inputs, as compared to hybrid cultivars which demand a strict crop production package. These two genotypes can also be of a great benefit in selection for high yield millet genotypes and/ or hybridization between them or with any other high yield millet genotypes. Component of variance and genetic parameters for different growth and yield characters were presented in Tables, 4 and 5. Phenotypic expression of the characters are result of interaction between genotypes and environment, Genotypic coefficient of variation (GCV) measures the range of variability in crop and also enables to compare the amount of variability present in different characters. The phenotypic coefficients of variation (PCV) estimates were higher than genotypic coefficients of variation (GCV) for all the characters studied among the fifteen pearl millet genotypes, indicated that the substantial influence of environment in the expression of these characters. Similar findings were observed in pearl millet by [7,8,21,20,22,23]. High GCVs and PCVs were observed for days to 50% flowering and days to maturity (based on combined result). These findings has earlier been reported by [24,17]. Heritability gives the information on the magnitude of inheritance of characters, while genetic advance is helpful in formulating suitable selection procedures. The information on heritability alone may not help in pointing characters for enforcing selection. Nevertheless, the heritability estimates in conjunction with predicted genetic advance will be more reliable. The estimates of heritability in broad sense and genetic advance for the studied characters were fluctuating at the two seasons. The differences in the magnitude of heritability would be attributed to the effect of the environment. Robinson et al. [25] attributed the change in heritability estimates in maize (*Zea mays* L.) to differential response of genotypes to the environment.

Table 2. Mean squares from the individual and combined analysis of variance for some growth and yield characters of pearl millet genotypes evaluated at Shambat during summer seasons of 2009 and 2010

Characters	Season 2009			Season 2010			(Combined)			
	(G) d.f =14	(E) d.f =28	C.V %	(G) d.f =14	(E) d.f =28	C.V%	(G) d.f =14	G×S Inter. d.f=14	(E) d.f =56	C.V%
Days to 50% flowering	257.75**	19.78	07.78	172.80**	30.34	09.77	342.8**	87.8**	25.10	08.82
Days to maturity	231.66**	25.91	06.66	364.17**	91.78	12.05	245.8**	350.00**	58.80	09.83
Plant height(cm)	290.56*	140.48	07.17	698.16 [n.s]	283.62	10.84	388.7 [n.s]	599.4**	225.10	09.34
Number of leaves	01.05 [n.s]	0.70	09.05	01.49 [n.s]	0.63	08.43	01.10 [n.s]	01.55*	0.72	08.74
Number of tillers	03.04 [n.s]	1.56	18.31	02.87 [n.s]	1.90	27.28	1.90 [n.s]	4.00*	1.70	22.16
Panicle length(cm)	05.18 [n.s]	4.96	13.65	18.94**	8.48	10.02	13.60*	15.00*	6.90	09.82
Number. of grains/plant	73.18**	27.88	25.90	1199369 8.57 [n.s]	961961. 22	31.18	829267. 5 [n.s]	1226836.4 [n.s]	771706.4	28.85
Grain yield/plant	8624105.35 [n.s]	581496.63	23.88	48.56 [n.s]	29.58	20.06	48.40 [n.s]	73.5*	225.10	60.96
1000 grain weight	01.52**	0.49	09.46	02.88 [n.s]	2.72	18.47	2. 6 [n.s]	01.8 [n.s]	1.60	15.52
Grain yield ton /hectare	00.35**	0.15	24.78	00.25 [n.s]	0.15	20.06	00.30*	00.30 [n.s]	0.20	22.17

*G:Genotype, S:Season, (G) and (E): Genotype and Error mean squares, d.f: Degree of freedom, CV%: Coefficient of variation. *: significant at the 0.05 probability level; **: significant at the 0.01 probability level; n.s: non-significant.*

Table 3. Means of individual and combined values for growth and yield characters of pearl millet genotypes evaluated at Shambat during summer seasons of 2009 and 2010

Genotypes	Days to 50% flowering			Days to maturity			Plant height (cm)			Numbers of leaves			Numbers of tillers		
	2009	2010	combined	2019	2010	combined	2009	2010	combined	2009	2010	combined	2009	2010	combined
ICMV 221	62.3	61.7	57.0	84.0	84.0	84.0	177.9	137.8	157.87	10.2	8.9	9.57	7.00	4.9	5.9
Ashana	54.7	50.3	58.17	80.7	80.7	80.7	174.5	137.5	155.97	9.8	9.8	9.82	7.3	5.2	6.23
ICMV 155	59.3	53.3	54.83	66.7	66.7	66.7	170.8	157.5	164.07	9.4	8.6	9.02	7.6	5.0	6.32
MCSRC	60	44.3	56.67	75.0	75.0	75.0	182.1	172.2	177.17	9.8	8.8	9.28	6.9	4.3	5.58
SADC (Long)	51.7	56	48.00	67.7	67.7	67.7	164.1	160.5	162.50	8.2	9.4	8.83	6.6	5.4	6.00
SADC (Togo)	49.7	48.7	52.83	71.7	71.7	71.7	167	148.6	157.77	9.2	9.4	9.32	6.9	6.6	6.78
Ugandi	55.7	50.7	52.17	71.3	71.3	71.3	159	151.6	155.52	95	8.9	9.17	7.3	5.8	6.53
Sudan II	43.3	50.3	47.00	69.3	69.3	69.3	150.9	160.3	155.63	8.3	8.8	8.53	5.9	4.6	5.23
Sudan III	51.7	56.7	51.00	70.0	70.0	70.0	172.6	150.0	161.32	9.2	9.0	9.12	6.2	6.7	6.45
MCNELC	56.3	56.7	56.50	82.7	82.3	82.7	171	134.7	152.85	10	9.6	9.83	6.3	5.6	5.95
Dembi Millet	51.7	48.3	58.67	83.3	83.3	83.3	145.8	160.9	153.35	8.9	9.4	9.15	5.9	5.8	5.87
Dembi Shangal Toby	51.3	67	49.83	78.3	78.3	78.3	165.5	154.2	159.83	9	9.0	9.03	5.4	5.0	5.17
Dembi Elfasher	76.7	65.0	71.83	100.0	100.0	100.0	161.5	194.2	177.73	9.1	11.3	10.20	5.6	3.8	4.68
Dembi Kabkaba	70.00	66	67.50	100.0	100.0	100.0	165.5	166.5	166.02	9.3	9.7	9.42	8.8	3.5	6.13
Dembi Sea	72.70	66.00	69.33	92.3	92.3	92.3	156.9	145.0	150.97	8.5	10.3	9.42	8.6	3.8	6.17

Contd. Table 3…

Genotypes	Panicle length			Numbers of grain /plant			Grain weight per plant (g)			1000grain weight (g)			Grain yield ton per hectare		
	2009	2010	combined	2009	2010	combined	2009	2010	combined	2009	2010	combined	2009	2010	combined
ICMV 221	25.50	29.60	27.52	3667.5	3622.1	3644.78	30.0	31.6	30.80	8.2	9.0	8.61	2.1	2.3	2.20
Ashana	27.00	29.60	28.87	2848.1	1914.9	2381.00	30.0	18.5	21.18	8.2	9.9	9.12	2.1	1.3	1.51
ICMV 155	26.50	30.70	27.73	3404.2	2600.6	3002.39	23.9	27.9	26.27	8.3	11.2	9.19	1.7	2.0	1.88
MCSRC	21.5	28.90	26.08	3204.8	3642.8	3423.84	24.6	26.0	24.61	7.2	8.8	7.94	1.8	1.9	1.76
SADC (Long)	24.1	30.20	27.52	2662.2	2779.4	2720.82	23.2	23.5	22.87	7.1	9.4	8.46	1.7	1.7	1.63
SADC (Togo)	23.4	27.10	25.28	3793.7	2583.1	3188.41	22.3	23.9	26.07	8.5	9.2	8.38	1.6	1.7	1.85
Ugandi	23.00	30.40	26.70	2750.5	3702.5	3226.48	28.2	33.1	28.64	7.5	8.9	8.14	2.0	2.4	2.05
Sudan II	26.2	26.10	26.13	2840.2	2851.6	2845.92	24.2	25.0	21.58	7.4	8.8	7.55	1.7	1.8	1.54
Sudan III	24.9	33.80	29.35	3202.9	3302.8	3252.85	18.2	28.8	27.10	8.3	8.7	8.40	1.3	2.1	1.94
MCNELC	22.8	29.30	26.03	3080.7	3351.5	3216.11	25.4	25.5	25.16	8.1	7.4	7.60	1.8	1.8	1.78
Dembi Millet	24.8	32.20	28.53	4303.9	2434.8	2919.35	24.9	22.9	23.89	7. 8	9.5	8.35	1.7	1.6	1.58
Dembi Shan Toby	23.8	29.40	26.62	2382.8	3109.6	2746.20	24.9	30.3	23.65	7.2	9.8	8.47	1.5	2.2	1.73
Dembi Elfash	21.2	26.60	23.87	1851	3369.7	2610.36	17	29.9	21.59	7.2	9.0	8.15	1.3	2.2	1.54
Dembi Kabka	26.8	25.30	26.07	2862	4456.8	3659.91	13.3	32.1	24.69	7.3	7.3	6.67	0.9	2.3	1.76
Dembi Sea	24.8	25.08	25.08	2202	2830.09	2830.09	17.3	21.09	21.09	6.0	7.34	7.34	1.2	1.51	1.51

Table 4. Estimates of individual and combined values of phenotypic (σ²ph) and genotypic (σ²g) variances and broad sense heritability h²(bs) for different characters of pearl millet evaluated at Shambat during summer seasons of 2009 and 2010

Characters	2009			2010			Combined		
=	σ² ph	σ² g	h²(bs)	σ² ph	σ² g	h²(bs)	σ² ph	σ² g	h² (bs)
Days to 50% flowering	99.1	79.3	0.80	77.83	47.49	0.61	130.96	105.9	0.81
Days to maturity	94.1	68.58	0.73	182.58	90.8	0.50	121.17	62.33	0.51
Plant height(cm)	190.51	50.02	0.26	421.80	138.17	0.33	279.66	54.52	0.19
Number of leaves	0.82	0.12	0.15	0.91	0.29	0.31	0.91	0.29	0.32
Number of tillers	2.05	0.49	0.24	2.22	0.32	0.14	1.79	0.6	0.34
Panicle length	6.76	1.41	0.35	0.18	0.03	0.17	9.13	2.21	0.24
Number of grains/plant	675132.82	93636.2	0.35	1039177.0	72260.7	0.07	790893.46	19187.04	0.03
Grain yield per plant	42.98	15.1	0.14	35.94	6.36	0.18	35.27	6.54	0.19
1000 grain weight	0.83	0.34	0.41	2.77	0.05	0.02	1.95	0.34	0.17
Grain yield ton /hectare	0.22	0.07	0.32	0.19	0.03	0.16	0.19	0.03	0.16

Table 5. Estimates of individual and combined values of phenotypic (PCV) and genotypic (GCV) coefficient of variations and Genetic advance (GA) for different characters of pearl millet evaluated at Shambat during summer seasons of 2009 and 2010

Characters	2009			2010			Combined		
	PCV	GCV	GA	PCV	GCV	GA	PCV	GCV	GA
Days to 50% flowering	17.42	14.59	16.41	15.56	12.22	11.09	18.56	16.69	19.06
Days to maturity	12.17	10.83	14.53	16.99	11.98	13.84	13.10	09.40	11.66
Plant height(cm)	8.33	4.27	7.47	13.21	7.56	13.86	12.13	05.36	06.72
Number of leaves	9.78	03.72	00.27	10.18	05.70	00.62	10.00	04.11	00.31
Number of tillers	21.01	10.31	00.71	29.49	11.21	00.44	27.30	04.98	00.09
Panicle length	10.46	4.85	01.11	11.90	06.42	02.08	10.22	05.03	01.51
Number of grains/plant	27.91	10.49	0.73	32.41	08.84	0.15	24.55	03.82	0.07
Grain yield per plant	29.65	17.57	04.75	22.11	09.30	02.19	18.77	08.09	02.27
1000 grain weight	12.34	07.92	00.77	18.66	02.62	00.07	15.45	06.50	00.51
Grain yield ton /hectare	29.76	16.47	00.29	22.17	09.45	00.16	19.06	08.26	00.17

The variation in heritability estimates for these characters was more obvious and would draw effort on the breeder to evaluate these characters in different environments. The combined results of the two seasons showed that high estimates of heritability in broad sense and genetic advance were scored for days to 50% flowering and days to maturity and lowest estimates for yield and yield components. High estimates of broad sense heritability coupled with higher genetic advance attained for days to 50% flowering and days to maturity indicated that these characters were under the control of additive genetic effects for their inheritance and they can be considered as favorable characters for pearl millet improvement through selection and this selection should lead to a fast genetic improvement. Similar findings were earlier observed by Abuali [8] in pearl millet and [26] in wheat. However, relatively high estimate of heritability with low genetic advance which were exhibited for number of tillers, indicated the presence of non additive gene action. Thus simple selection for this character will not be effective. In such situation recombination breeding may be give better response for improvement of millet.

4. CONCLUSIONS

Based on results of this study, it could be concluded that there was considerable amount of variability present in the genotypes. High estimates of broad sense heritability coupled with higher genetic advance were attained for days to 50% flowering and days to maturity. On the other side relatively high estimates of heritability with low genetic advance were exhibited for number of tillers per plant. This variation in the studied evaluated criteria could be effectively manipulated with appropriate breeding methods and programs at the Sudan to develop improved varieties and hybrids for use by farmers. The genotypes ICMV155 and SADC (long) scored the most minimum days to maturity and a moderate values of yield per ton/ha, indicating the availability of using them at drought areas of the Sudan. The superiority of the genotypes ICMW221 and Ugandi over the other evaluated millet genotypes in yield (t/ha) suggests their adoption as ones of the high yielding cultivars at the field conditions of the Sudan. However, further investigation is needed for studying the role and contribution of the stability of different millet characters under a range of environments regarding the irrigated and water scarcity schemes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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