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Agronomic performance of improved pearl millet cultivars in southern Chad

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ABSTRACT

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a vital staple food crop for poor people in dry-land areas. It plays an important role on human consumption in South of Chad where farmers always used landraces with a little adoption of improved cultivars. Evaluation of agronomic performance of inbred cultivars at research station is important to identify the high performance genotype to be released as new cultivars in farmer area. So, five improved varieties associated with a well-known variety from research were evaluated for ten agronomical traits. Experiment was carried out during rainy season of 2019 at the research station of Bébédjia, South of Chad. The results of the analysis of variance of assessed traits showed high significant differences ($p < 0.001$) for spike girth and slight significant difference ($p < 0.05$) for days to flowering, thousand seeds weight and productive tillers per plant. An important positive and significant correlations were also noted between day to flowering and number of grains per plot ($r = 0.7$; $p < 0.01$), seed weight per plot (0.684; $p < 0.01$) and potential grain yield ($r = 0.684$; $p < 0.01$). Moreover, three cultivars with an important potential grains yield namely ICMV167006 (4.99 t.ha⁻¹), ICMV 167005 (4.68 t.ha⁻¹) and ICMV177111 (3.98 t.ha⁻¹) were identified. Otherwise, for the days to flowering, the cultivars ICMV167006 (73.67 days), ICMV 167005 (75.33 days) are earlier than ICMV 177 111 (78.67 days). These cultivars could be proposed to be released in farmer area as novel cultivars.

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Keywords: Pearl millet, agromorphological characterization, cultivars, southern Chad.

INTRODUCTION

Pearl millet ($2n = 2x = 14$) belongs to a highly heterozygous group that contains both wild and cultivated forms (Hancock, 1992). It is one of the most important staple cereals for subsistence farmers living in semiarid tropics of Africa and Asia., due to its high tolerance to drought, high temperatures, saline and marginal soils, and high capacity to buffer

variable environmental conditions (Hausmann et al., 2012; 2016; Das, 2017). Approximately, 500 million people depend on pearl millet and it is currently grown on 28 million hectares throughout the world (Siddaiah et al., 2017). It is cultivated in diverse environments with harsh climate, frequent drought events and poor soil fertility where other cereal crops generally fail (Kumari

et al., 2016; Sangwan et al., 2019). Pearl millet production is also hampered by several biotic constraints such the Millet Head Miner (*Heliocheilus albipunctella* De Joannis) and Downy mildew disease caused by *Sclerospora graminicola* (Sacc) Schroët (Halilou et al., 2017 ; Zoclanclounon et al., 2018 ; Kadri et al., 2019 ; Oumarou et al., 2020).

In Chad, pearl millet is the second important cereals after sorghum, grown in 1.23 million hectares which represent about 37% of total area planted under cereals. On 2018, the total production is 774,498 tones grains per year and occupies 27% of total cereals production of the country (DSA, 2018). Pearl millet grains are used for human consumption and for animal feed. It has higher protein, fat and balanced amino acid for human nutrition than that of wheat, maize or polished rice (Sawaya et al., 1984; Khairwal et al., 1999). According to Izge et al. (2007), it is a nutritious crop, producing almost all the required nutrients needed by humans. Filardi et al. (2005) reported that pearl millet produces higher protein levels than maize and have approximately 85% of energy content of maize.

The average productivity of pearl millet in Chad for the ten last years has been 525.44 kg.ha⁻¹ for a maximum productivity of 636.51 kg.ha⁻¹ (DSA, 2018). Several reasons are related to this fall of production in particular the exclusive use of landraces, by the producers. Even today, West and Central Africa farmers growing this cereal predominantly use pearl millet landraces (Busso et al., 2000; Dingkuhn et al., 2006). Improved varieties were released in farmer area by research during long time, however very little were adopted by them. Improving production of pearl millet while maintaining its production stability is crucial for food security for poor African smallholder farmers cropping under rain-fed farming systems (Pucher et al., 2015). According to Acquah (2012), yield is the ultimate goal of plant breeding programs.

Assessment of news genetic material developed by breeding program is essential to identify a genotype to be released as cultivar to producers (Ceccarelli, 2009). The first stage to evaluate the news genotypes for identify superior genotypes is to study their agronomic performance at the research station, associate with a local existing variety more appreciated by farmers in this locality. Therefore, the current study aimed (i) to evaluate of level of agromorphological variability, (ii) to determine the agronomic performance and potential yield of assessed plant material and (iii) to select the best pearl millet cultivars as potential new cultivars to be release in southern Chad.

MATERIALS ET METHODS

Plant material

The experiment material included five pearl miller cultivars provided by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Niger and S 42, one superior cultivar well-known and more cultivated in environment of test of Bébédjia and considered as control in this test (Table 1).

Experiment design and cultural practices

The experiment was conducted under field conditions, at the experimental station of Bébédjia (9°55'N and 15°8'E), at National Agricultural Research Institute of Chad (Institut tchadien de Recherche Agronomique pour le Développement, ITRAD), located in Logone Oriental region. It was set in a randomized block design with five replications and six treatments. Plot consisted of five rows of 5-m length, spaced 0.8-m apart.

Seeds were hand sowed 80 cm apart in the furrows, covered with soil using hoes and the soil compressed over the seed by walking several times over the seed rows. Sowing was realized on July 22, 2019 and fertilizer (NPK 10-10-10) was applied the same date at the rate of 100 kg.ha⁻¹. At growth stage (August 08, 2019), plants were thinned to one plant per hole

after weeds were removed by rotary hoe. Three weeks after the first management of weed control (August, 28 2019) the second removing weeds was done.

Data collection

Data are collected over all plant development stages. At the reproductive stage, flowering time was estimated as the number of days between sowing and full stigma emergence on main spike of 50% of plant on plot. The percentage of downy mildew-infested plants was recorded to determine the susceptibility of cultivars assessed. Total tillers per plot were determined by accounting the number of tillers in plot and after physiological maturity, the productive tillers per plant were determined counting the number of tillers for each. Before harvesting the plant height and spike girth were measured on 10 randomly selected plants on plot. After harvested and drying, the spikes of three central rows were gathered and seed weight per plot was measured using accuracy balance. The seed

weight per plot was used to estimate the potential grain yield.

Data analysis

Data were subjected to the descriptive statistics (mean, standard deviation, coefficient of variation) and analysis of variance (ANOVA) using XLSTAT 2016 edition software. In addition, principal component analysis (PCA) was conducted after standardization to mean of zero and variance of one. The Pearson’s correlation coefficients between each pairs of traits were performed. The heritability (h^2) was categorized as suggested by Johnson et al. (1955) using GenStat 12th edition software. In addition, means separation test of Newman-Keuls at the 5% threshold was performed to determine the significance of the means differences between ICRISAT pearl millet cultivars for the discriminating traits and to compare them to the tester.).

Table 1: pearl millet cultivars used as plant material.

Cultivar code	Cultivar name	Origin
V1	CHAKTI	ICRISAT-Niger
V2	ICMV 160 01	ICRISAT-Niger
V3	ICMV 167 005	ICRISAT-Niger
V4	ICMV 167 006	ICRISAT-Niger
V5	ICMV 177 111	ICRISAT-Niger
V6	S 42 (Tester)	Bébédjia (Chad)

RESULTS

Weather condition

During the growing period of pearl millet, accounting between July and end of October, average of monthly relative humidity ranged from 85.16 (July) to 80% (October) and decreased rapidly to 67.47% in November (Figure 1). There was similarity between the average monthly and noon monthly relative humidity. At that time, the monthly morning relative humidity varied from 92.77 (July) to 93.68% (October) and decreased in November to 86.1%. However monthly noon relative humidity varied from 71.68 (July) to 66.52% (October) and decreased in drastic manner to 43.2% in November. During the dry season from November to March relative humidity was down.

The growing season during this study was characterized by monthly minimal air temperature, which ranged from 28.09 (April) to 23.87 °C (October) and the maximal air temperature which varied from 41.61 (April) to 34.54% (October). During the growing stage of the trial, from sowing (July) to harvesting (October), minimal air temperature ranged from 23.87 to 23.64 °C and maximal air temperature varied from 34.54 to 33.89 °C. The rainfall in growing season was 1180.6 mm, started in April with 24.4 mm and ended in October with 107.2 mm. August was the high wet month with 410.9 mm following by July with 243.6 mm. During the wet period of August minimal and maximal air temperatures were the most down for the growing season, respectively 23.55 and 32.39 °C (Figure 2).

Variation of assessed traits

The results of analysis of variance of assessed traits (Table 2) showed high significant differences ($p < 0.001$) only for spike girth and slight significant difference ($p < 0.05$) for days to flowering, thousand seeds weight and productive tillers per plant. For the rest of traits, the difference among cultivars was non-significant ($p > 0.05$). Coefficient of variation

(CV) varied from 8.57% for days to flowering to 38.11% for productive tillers per plant. It was higher ($CV > 33\%$) for traits related to yield, especially for productive tillers per plant (38.11%), seeds weight per plot (33.95%) and potential grain yield (33.95%). So the yield and yield component were more variables. For vegetative tillers per plot (17.68%), plant height (15.87%) and thousand grains weight (12.96%), the CV were slight variables and for the days to flowering (8.57%), variability was less variables.

Potential grain yield varied from 2.16 to 4.99 t.ha⁻¹, with an average of 3.57 t.ha⁻¹. The flowering time ranged from 62 to 78.67 days, with an average of 73.17 days showing that, the assessed cultivars were early flowering varieties. The plant height averaged 197.9 m/plant and ranged from 169.67 to 253.33 m/plant. Number of grains per unit area ranged from 17067 to 51862 grains per plot with an average of 33702 grains per plot. Seeds weight ranged from 173 to 399.33 g/plot with a mean of 285.5 g/plot. The mean of spike girth was 8.8 cm; the lowest was 6.93 and however a maximum spike girth was 11.8 g. The vegetative tillers ranged from 76.67 to 123 per plot with an average of 94.9 and productive tillers ranged from 6.67 to 20.07 per plant with an average of 12.29 per plant. Downy mildew susceptibility ranged from 6 to 11.33% among cultivars with an average of 8.72%.

Relationship between traits

Significant correlations between different quantitative traits assessed were revealed by Pearson correlation coefficient (Table 3). Spike girth was positively correlated only with thousand seed weight ($r = 0.911$; $p < 0.001$) but negatively correlated with each of the rest of the traits. Thus, thousand seed weight was negatively correlated with seed weight per plot ($r = -0.766$; $p < 0.01$), potential grain yield ($r = -0.766$; $p < 0.01$), plant height ($r = -0.785$; $p < 0.01$), productive tiller per plant ($r = -0.746$; $p < 0.01$), vegetative tiller per plot (r

= -0.893; $p < 0.001$) and number of days to flowering ($r = -0.608$; $p < 0.01$). In addition, day to flowering was positively correlated with number of grains per plot ($r = 0.7$; $p < 0.01$), seed weight per plot (0.684; $p < 0.01$) and potential grain yield ($r = 0.684$; $p < 0.01$). Vegetative tiller per plot positively contributed to productive tiller per plant ($r = 0.882$; $p < 0.001$). Plant height increase productive tillers per plant ($r = 0.879$; $p < 0.001$) and vegetative tillers per plot ($r = 0.882$; $p < 0.001$).

Analysis of association between traits

Principal component analysis (PCA) reduces the dimensions of multivariate data by removing inter-correlations among the traits being studied, thereby enabling multidimensional relationships to be plotted on two or three principal axes. The PCA based on eight traits (excluding yield) revealed that the first three principal components provided a reasonable summary of data and explained 93.727% of variance. The three PC explained 53.148%, 23.356% and 17.223% of the phenotypic variation, respectively for PC1, PC2 and PC3 (Table 4). Thousand seeds weight and spike girth length had relatively large positive influence on the first component. Conversely, number of vegetative tillers, number of grains per plot, seeds weight per plot, potential grain yield, and plant height had negative influence. The second component separated number of days to flowering, number of grains per plot, seed weight per plot and potential grains yield (yield and components) to plant height and number of productive tiller. The third PC axis was negatively influenced by flowering time, vegetative tillers and plant height.

Performance analysis for assessed cultivars

Average performance analysis of assessed cultivars for yield and yield

component showed high difference among cultivars (Table 5). Variance component analysis indicated significant main effects of accessions for number of days to flowering ($p = 0.039$), thousand seeds weight ($p = 0.011$), number of productive tillers ($p < 0.016$) and spike girth ($p = 0.0001$). For seeds weight per plot, potential grains yield and plant height there was non-significant difference among cultivars.

The mean of flowering time ranged from 62 to 78.67 days. CHAKTI had an earlier flowering time (62 days) and the rest of the cultivars had more than 70 days for flowering time. ICMV 160 01 (V2) had a heavier thousand seeds weight (10.43 g) followed by CHAKTI (9.83 g) and the cultivar ICMV 167 006 showed the smaller thousand seeds weight (7.5 g). The average plant height was highest for tester S42 (253.33 cm), followed by ICMV 167 006 (210.33 cm) and ICMV 167 005 (201 cm). The tester S42 produced more productive tillers plant (≈ 20) than the new cultivars assessed.

Regarding the seed weight per plot, the ICMV 167 006 produced higher seed weight per plot (399.33 g), followed by ICMV 167 005 (374.67 g) and ICMV 177 111 (318.67 g). The tester S42, used to compare the new cultivars produced only 272 g seeds per plot. Considering the number of grains per plot, cultivar ICMV 167 006 produced higher number of grains per unit of area (51862 grains), followed by ICMV 167 005 (43395 grains) and S 42 (36620 grains). The average potential grain yield was higher for ICMV 167 006 (V4), which produced $4.99 \text{ t}\cdot\text{ha}^{-1}$, followed by ICMV 167 005 (V3 = $4.68 \text{ t}\cdot\text{ha}^{-1}$) and ICMV 177 111 (V5 = $3.98 \text{ t}\cdot\text{ha}^{-1}$). These three cultivars were identified more superior than the existing variety more produced by the farmers and used as a tester.

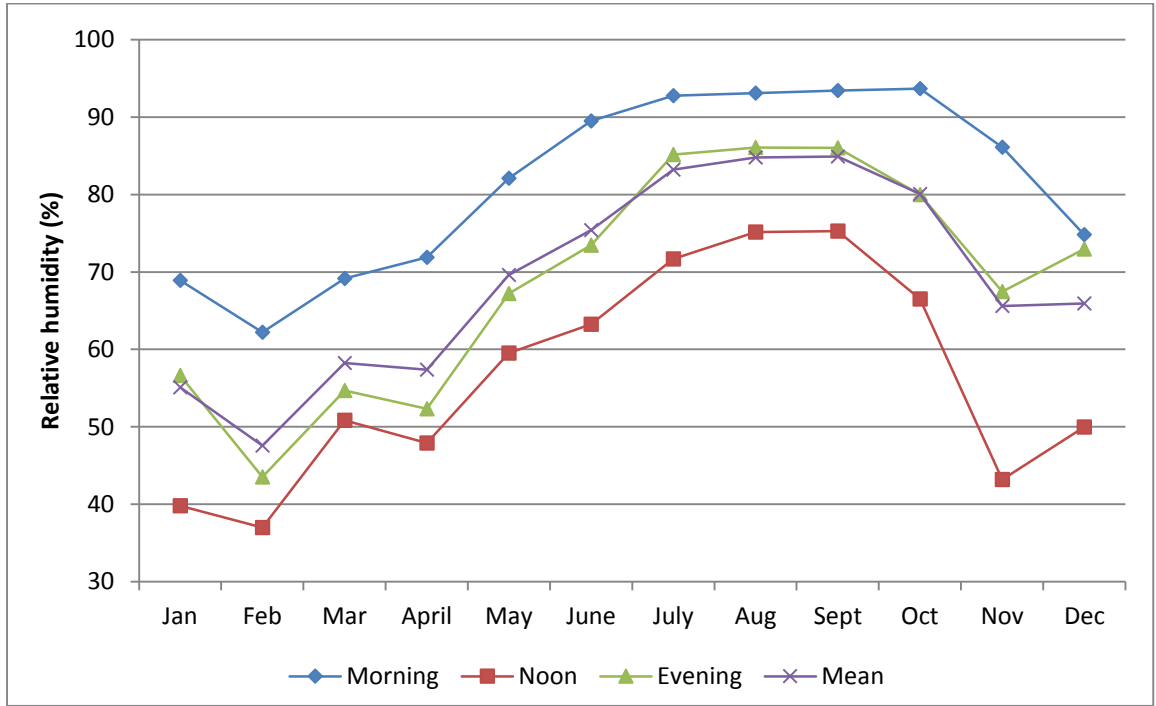


Figure 1: Air humidity relative during year of experiment.

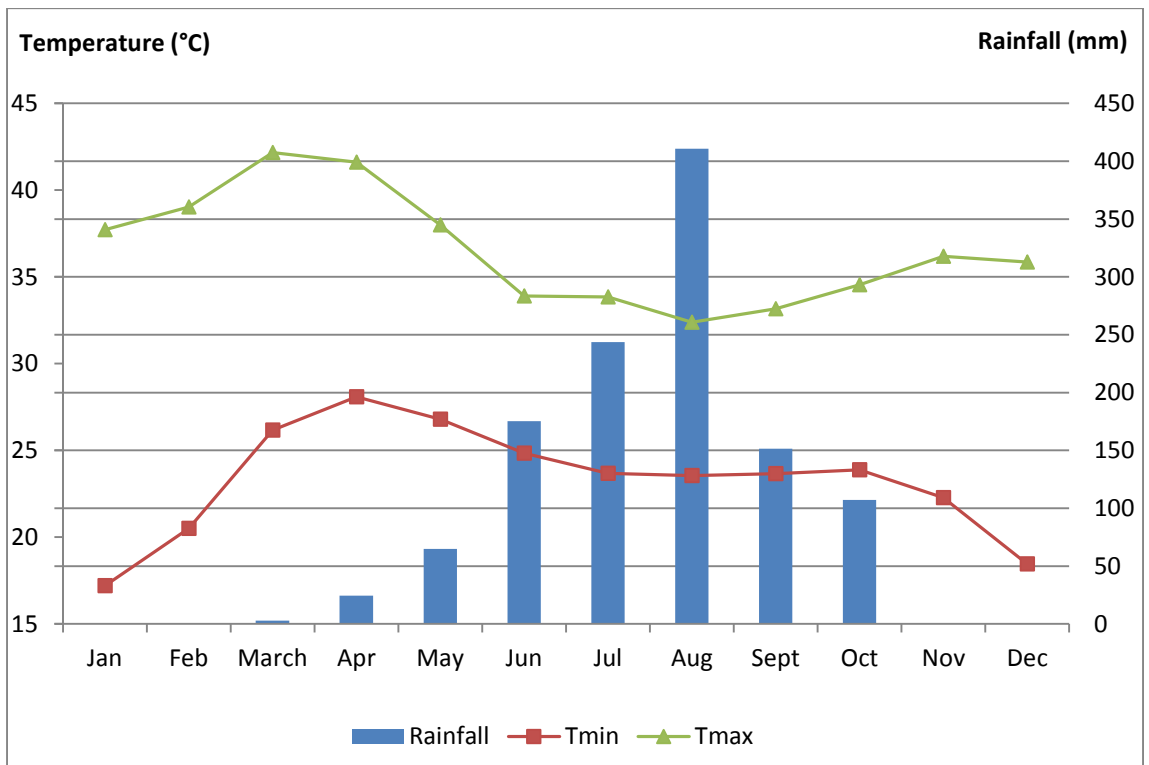


Figure 2: Air temperature and rainfall condition during year of experiment.

Table 1: Results of analysis of variance and broad sense heritability of assessed traits.

Traits	Minimum	Maximum	Mean	CV (%)	H ² (%)
Days to flowering (day)	62	78.67	73.17±6.27	8.57*	70.48
Downy mildew-infested plants (%)	6	11.33	8.72±1.95	22.34 ^{ns}	27.83
Thousand grains weight (g)	7.5	10.43	8.89±1.15	12.96*	79.52
Seeds weight per plot (g)	173	399.33	285.5±96.9	33.95 ^{ns}	37.07
Number of grains per plot	17067	51862	33702±14007	41.56 ^{ns}	37.07
Potential grain yield (t.ha ⁻¹)	2.16	4.99	3.57±1.21	33.95 ^{ns}	37.07
Plant height (cm)	169.67	253.33	197.9±31.4	15.87 ^{ns}	60.03
Vegetative tillers per plot	76.67	123	94.9±16.8	17.68 ^{ns}	65.23
Productive tillers per plant	6.67	20.07	12.29±4.68	38.11*	77.46
Spike girth (cm)	6.93	11.8	8.8±1.76	20.00 ^{***}	98.18

Significant: * p<0.05; *** p<0.001; CV: coefficient of variation; H²: heritability

Table 2: Pearson's correlation coefficient among agronomical assessed traits.

Variables	DFL	VTL	TSW	NGU	SWP	PGY	PHT	PTL
VTL	0.611**							
TSW	-0.608**	-0.893***						
NGU	0.700**	0.621**	-0.880***					
SWP	0.684**	0.461*	-0.766**	0.978***				
PGY	0.684**	0.461*	-0.766**	0.978***	1***			
PHT	0.560*	0.916***	-0.785**	0.480*	0.311	0.311		
PTL	0.222	0.882***	-0.746**	0.352	0.172	0.172	0.879***	
SPG	-0.536**	-0.752**	0.911***	-0.887***	-0.830***	-0.830***	-0.517*	-0.587*

DFL: days to flowering; VTL: vegetative tiller; TSW: thousand seeds weight; NGU: number of grains per unit area; SWP: seed weight per plot; PGY: potential grain yield; PHT: plant height; PTL: productive tiller; SPG: spike girth; Significant: * p<0.05; ** p<0.01; *** p<0.001

Table 3: Principal component (PC) matrix explaining loading of variables.

Axis	PC1	PC2	PC3
Eigenvalue	7.441	3.270	2.411
% of total variance	53.148	23.356	17.223
Cumulative variance (%)	53.148	76.504	93.727
Days to flowering	-0.248	0.226	-0.360
Vegetative tiller	-0.297	-0.197	-0.275
Thousand seeds weight	0.359	0.081	0.038
Number of grains per unit area	-0.338	0.294	0.090
Seed weight per plot	-0.317	0.256	0.094
Potential grain yield	-0.317	0.256	0.094
Plant height	-0.265	-0.278	-0.246
Productive tiller	-0.229	-0.406	-0.149
Spike girth	0.330	-0.023	0.011

Table 4: Results of Neman Keuls average separation test of the traits of pearl millet cultivars assessed.

	DFL	TSW	SWP	NGP	PGY	PHT	PTL	SPG
ICMV 167 006	73.67 ab	7.5 b	399.33 a	51863	4.99 a	210.33 a	14.27 ab	6.93 d
ICMV 167 005	78.67 a	8.7 ab	374.67 a	43395	4.68 a	201 a	10.53 b	8.47 c
S 42	78.67 a	7.73 b	272 a	36621	3.4 a	253.33 a	20.07 a	7.8 c
ICMV 177 111	75.33 ab	9.13 ab	318.67 a	35909	3.98 a	169.67 a	9.13 b	7.93 c
CHAKTI	62 b	9.83 ab	173 a	17358	2.16 a	177.33 a	13.07 ab	9.87 b
ICMV 160 01	70.67 ab	10.43 a	175.33 a	17066.6	2.19 a	176 a	6.67 b	11.8 a
Pr > F	0.039	0.011	0.236	0.169	0.236	0.090	0.016	0.0001
Significant	Yes	Yes	No	No	No	No	Yes	Yes

DFL: days to flowering; TSW: thousand seeds weight; NGP: number of grains per plot; SWP: seed weight per plot; PGY: potential grain yield; PHT: plant height; PTL: productive tiller; SPG: spike girth; a, b, c = the values followed by the same letters are not significantly different at the threshold of 5%.

DISCUSSION

Assessment of five new cultivars of pearl millet in southern Chad compared with an existing variety as control was carried out to identify superior genotype that can be released as a new cultivar to farmers for commercial production. The result of this study indicated that during the growing season of experiment, average relative humidity ranged from 85.16 (July) to 80% (October). Minimal air temperature varied from 23.87 to 23.64 °C and maximal air temperature ranged from 34.54 to 33.89 °C. The rainfall was 1180.6 mm; started in April with 24.4 mm and ended in October with 107.2 mm. The cultivars were sowed July 22, 2019 and during the growing period of cultivars weather was good with regular rain and nice minimal and maximal air temperature. These indicated that experiment was realized in the optimum weather conditions for the development of pearl millet.

Large variations between cultivars were observed for some traits assessed through analysis of variance. For days to flowering, thousand seeds weight, productive tillers and spike girth highly and significant variation among cultivars were observed. For number of grains per plot, seeds weight per plot, potential grain yield, plant height and vegetative tillers, although the difference between cultivar was not significant, these traits showed high coefficients of variation (>33%) expressed dispersal of cultivars values of these regarding mean value. According to Acquah (2012), coefficient of variation is a measure of the relative variability of given populations. Number of productive tillers showed highly variation (CV: 38.11%). Kumari et al. (2016) and Gapili et al. (2019) reported similar results. They obtained a CV of 38% and 43.3% respectively for the same trait. Previous study realized on pearl millet landrace from South of Chad found that all assessed traits except total tiller per plant revealed high significant difference between twelve landraces (Gapili et al., 2019). Govindaraj et al. (2010) found highly significant differences among accessions from India for almost assessed traits except for number of productive tillers and days to maturity. Evaluation of downy mildew

susceptibility showed that an average of 8.72 % was attacked. Pucher et al. (2015) on West and Central African pearl millet accessions registered a higher value of 17.49%. Study realized on Indian pearl millet found that out of 221 accessions, 107 accessions were free from downy mildew disease significantly (Kumari et al., 2016). Pearl millet cultivar grew even less than 254 cm with a mean value of 197.9 cm with the taller plants from tester S42. All assessed cultivars were characterized by early and medium flowering time (62 to 78.67 days), good productive tillers capacity (from 6.67 to 20 tillers).

Study noted marked relationships between the various traits measured. Flowering time is positively correlated with yield and yield component. According to Gapili et al. (2019) number of days to flowering is highly positively correlated with weight of main spike and grain weight per main spike (yield components). Contrariwise, study realized by Bidinger et al. (1987) found a strong negative correlation between flowering time and grain yield when pearl millet receives terminal drought stress. According to Pucher et al. (2015), the correlation between flowering time and grain yield varied across test environments. Seeds weight per plot and number of seed per plot enhanced significantly the potential grain yield. According to Araus et al. (2009), the two main yield components are the number of grains per unit area and the averaged individual grain weight.

PCA reduces the number of variables to be used for prediction and description (Acquah, 2012). These procedures are used to reduce the number of variables and to detect structure in the relationships between these variables (Karsai et al., 2010). The current study allowed seeing strong association among flowering time, vegetative tiller and plant height on the one hand. On the other hand, high relation was observed among number of vegetative tiller, number of grains per plot, seed weight per plot, potential grain yield, and plant height.

In this study the existing variety used as a control produced taller plant (253.33 cm) than the new cultivars. According to Ceccarelli

et al. (2000) and Annicchiarico et al. (2005), reduced plant height has been one of the major causes of lack of adoption of modern varieties in a number of crops grown by farmers in developing countries as animal feed and where the way in which total biomass is portioned is irrelevant. In Chad, farmers used the pearl millet straw to build roofs and for animal feed. Regarding thousand grains weight, cultivar ICMV 160 01 showed highest value (10.43 g), followed by CHAKTI (9.83 g) and ICMV 177 111 (9.13 g). The smaller thousand grains weight was for ICMV 167 006 which had 7.5 g. study realized on pearl millet accessions from West and Central Africa found the thousand grains weight ranged from 4.5 to 13.5 g with an average of 8.7 g (Sattler et al., 2017). Cultivar ICMV 167 006 produced higher seed weight per plot with higher number of seeds per plot (399.33 g and 518623 respectively), followed by ICMV 167 005 (374.67 g and 43395) and ICMV 177 111 (318.67 g and 35909). According to Araus et al. (2009) the number of grains per unit area and the averaged individual grain weight are the two main yield components, these three cultivars mentioned above with higher seed weight per plot and higher number of grains per plot were the higher potential grains yield. Then, cultivar ICMV 167 006 produced the higher potential grains yield (4.99 t.ha⁻¹), followed by ICMV 167 005 (4.68 t.ha⁻¹) and ICMV 177 111 (3.98 t.ha⁻¹).

In fact, the average productivity of pearl millet in Chad for the ten last years has been 525.44 kg.ha⁻¹ for a maximum productivity of 636.51 kg.ha⁻¹ (DSA, 2018). In other countries like India, average productivity of pearl millet was 930 kg.ha⁻¹ (AICPMIP, 2017). Some studies in India showed that a hybrid variety of pearl millet produced 1270 kg.ha⁻¹ (Gupta et al., 2015). Then these three cultivars with higher potential grains yield were more productive than average production of pearl millet in Chad. They could be released as new cultivars to farmers for commercial production. Once new and potential promising lines are identified by agricultural research, it is essential to commercialize and make their seeds available to farmers

Conclusion

The results of our study indicated that days to flowering, thousand seeds weight, productive tillers and spike girth showed significant difference among cultivars. Flowering time ranged from 62 to 78.67 showing that assessed cultivars had earlier flowering time. Pearson's coefficient of correlation showed high relation between day to flowering and grains potential yield and yield components. Among the news assessed pearl millet three cultivars ICMV 167 006 (4.99 t.ha⁻¹), ICMV 167 005 (4.68 t.ha⁻¹) and ICMV 177 111 (3.98 t.ha⁻¹) produced higher grains yield potential than the well-known cultivar (S42) which reached 3.4 t.ha⁻¹ grains yield potential. These cultivars could be assessed in farmer trial to associate producers in the definitely choice of the new cultivars to be released in farmer area.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

GN, NS, and ND designed the research. GN and NS wrote the manuscript and performed the experiments. GN, NS and MAH, analyzed the data. All authors read and approved the final manuscript.

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