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Characterization of Sudanese pearl millet germplasm for agro-morphological traits and grain nutritional values

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Abstract

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important staple cereal cultivated in the arid and semi-arid tropics of Asia and Africa, regions severely affected by malnutrition. Knowledge about the extent of genetic variability and patterns of agro-morphological variation in local germplasm from a target region is an important prerequisite for efficient crop improvement. To assess the potential of Sudanese pearl millet landraces as sources of desirable traits for pearl millet improvement including biofortification, a total of 225 accessions were evaluated in Sudan at three locations for agro-morphological traits and at one location for grain mineral nutrient contents (Fe, Zn, Ca, P, K, Mg, Mn, S, Na, Cu and β-carotene). Genetic variation was highly significant, but relatively limited for some agro-morphological traits (62–78 d to flowering, 119–188 cm plant height and 16–34 cm panicle length), pointing to the potential usefulness of a targeted diversification for these traits. Self-pollinated grain micronutrient contents showed a wide variation: 19.7–86.4 mg/kg for Fe and 13.5–82.4 mg/kg for Zn. Significant and positive correlations among most of the nutritional traits were observed; therefore, enhancement of the concentrations of some nutrients will lead to the improvement of other related nutrients. No significant associations were observed between the nutritional and agro-morphological traits, indicating good prospects for simultaneous improvement of both trait categories. No clear patterns of geographic differentiation for specific traits were detected for the Sudanese pearl millet. Nutrient-rich accessions were identified and those with acceptable agro-morphological traits are encouraging materials for future pearl millet biofortification programmes in Sudan.

Keywords: biofortification; genetic diversity; geographic differentiation; pearl millet; phenotypic clusters; Sudan

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important crop in arid and semi-arid tropics of Asia and Africa, where it is cultivated for food, fodder and building materials. As a staple cereal, it represents the most common source of energy and micronutrients for millions of the world's poorest crop-livestock producers (FAO-ICRISAT, 1996; Rao *et al.*, 2006). The West African Sahelian belt from the Sahara Desert to the Sudanian savanna zone is the centre of origin for pearl millet, which was domesticated some 4000 to 4800 years ago (Harlan, 1971; Tostain, 1992; Oumar *et al.*, 2008; Manning *et al.*, 2011; Clotault *et al.*, 2012).

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In the western region of Sudan, pearl millet is grown as a grain crop for preparing fermented or unfermented pancake ('Kisra'), stiff ('Aseda') or thin ('Nasha') porridges, and local alcoholic beer ('Marisa') or non-alcoholic ('Abrei' or 'Hullu-murr') beverage. Since the diets in this region are characterized by a strong prevalence of pearl millet, high contents of bioavailable micronutrients in pearl millet grain could significantly contribute to the reduction of malnutrition.

Access to well-characterized, adapted germplasm and knowledge about the geographic patterns of genetic variation for various traits in a target region are important prerequisites for successful plant breeding (Haussmann *et al.*, 2004). Understanding the structure of diversity and identification of distinct materials with complementary traits for crossing provides the foundation for effective and sustained pearl millet population breeding, synthetic and hybrid development, based on the concept of heterotic groups in this allogamous crop. Identification of contrasting parental materials to enhance heterozygosity or to optimize genetic heterogeneity in a hybrid population can also be a means of enhancing yield stability in variable and changing climates (Haussmann *et al.*, 2007, 2012).

In the context of developing fortified foods for low-income consumers, different studies have revealed a wide range of genetic diversity for pearl millet grain micronutrient contents, with Fe and Zn contents reported to be highly heritable (Abdalla *et al.*, 1998; Malik *et al.*, 2002; Velu *et al.*, 2007, 2008a; Ashok Kumar *et al.*, 2010; Govindaraj *et al.*, 2011, 2013). However, little information is available on mineral micronutrient contents and β-carotene concentrations in the Sudanese pearl millet.

Nutrient density traits must be transferred to high-yielding cultivars. To have a high adoption and maximum impact, high-yielding genotypes with excellent grain quality are needed (Graham *et al.*, 2001). These efforts are called 'biofortification' because they refer to the bioavailable micronutrient content of food crops enhanced through genetic improvement.

The overall goal of the present study was to contribute to the characterization and use of Sudanese pearl millet germplasm. Specific objectives were to:

(1) characterize pearl millet germplasm from Sudan for agro-morphological traits as well as for 11 macro- and micronutrient contents (including Fe, Zn, Ca and β-carotene);
(2) study the relationships between agro-morphological and grain nutrient traits and the geographic distribution of pearl millet phenotypic diversity in Sudan;
(3) group the accessions into morphologically distinct clusters;
(4) identify promising genotypes with high yield and high grain nutrient contents for direct cultivation and/or use as parents in future crop improvement programmes.

Materials and methods

A total of 225 pearl millet accessions were used in this study. The material includes 100 accessions of pearl millet landraces or local varieties collected from different ecological zones and breeders' lines from Western Sudan, 115 landraces selected from the Genetic Resource Unit at Agricultural Research Corporation (ARC), Sudan, and ten landraces or improved varieties provided by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Niger, West Africa (Table S1, available online).

Field trials

The 225 accessions were evaluated under field conditions at three distinct locations (Wad Medani, El Fasher and El Obeid) in Sudan during the 2010 rainy season (Table S2, available online). The experiments were arranged in 15 × 15 lattice designs with three replications. Each accession was sown on ridges (Wad Medani) or flat lines (El Fasher and El Obeid) in two-row plots. The rows were 2 m long and spaced 0.75 m apart with an intra-row spacing of 0.50 m between hills for the El Fasher and El Obeid sites, and 2 m long and spaced 0.80 m apart with an intra-row spacing of 0.20 m between hills for the Wad Medani site. Surface irrigation was performed every 2 weeks at the Wad Medani site and fertilizer in the form of urea at a single dose of 80 kg/ha was applied 4 weeks after sowing. While the treatments such as spacing or irrigation partially differed among the sites, the testing conditions represented the respective recommendations for pearl millet cultivation at each location.

Data collection

Agro-morphological traits

The following traits were recorded from five randomly labelled plants in each plot: days from sowing to 50% flowering (when 50% of plants showed stigma emergence on the main tiller); plant height (cm); productive tillers/plant (n); plant population/m^2 (n); days from sowing to 75% maturity; panicle length (in cm, length of the panicle on the main axis measured at the dough stage); panicle exsertion (in cm, distance between the ligule of the flag leaf and the base of the panicle); panicle width (in mm, maximum diameter of the panicle measured at the dough stage); synchrony of panicle maturity (scale 1 to 9: 1 = non-synchronous and 9 = highly synchronous); panicle density (scale 1 to 9: 1 = very loose and 9 = highly compact); grain colour (classes: ivory, cream, yellow, grey, deep grey, grey brown, brown, purple and purplish black) (IBPGR and ICRISAT, 1993); 1000-seed weight (g); grain yield (weight of the dry threshed grain per plot, converted to g/m^2); dry forage yield (weight of the dry stover and panicle debris after threshing per plot, converted to g/m^2). The harvest index (%) was computed as the percentage ratio of grain weight to total above-ground dry matter weight for each plot, with the total above-ground dry matter weight being the sum of dry stover and unthreshed panicle weight).

Nutritional traits

Nutrient content analyses were performed only with grains from the Wad Medani site. To avoid contamination during the pollination period and to ensure that pure seeds from each accession were harvested, ten panicles per plot were bagged before flowering with Kraft paper bags. Samples of 1 g from the self-pollinated seeds of each accession from the Wad Medani site were analysed in the laboratory (State Institute for Agricultural Chemistry, University of Hohenheim, Germany), for macro- and micronutrients such as iron (Fe), zinc (Zn), calcium (Ca), sodium (Na), magnesium (Mg), manganese (Mn), copper (Cu), sulphur (S), phosphorus (P) and potassium (K). First, plant samples were digested according to the method developed by VDLUFA (2009). Second, nutrient measurements were conducted using an inductively coupled plasma optical emission spectrometer according to the procedure described by VDLUFA (2009). Furthermore, analysis of β-carotene concentrations was carried out in the laboratory (Institute of Biological Chemistry and Nutrition, University of Hohenheim, Germany), following the method outlined by Epler *et al.* (1993).

Statistical analysis

All statistical computations were performed using GenStat software, version 14 (VSN International, 2011; Payne *et al.* 2011). Location-wise and combined analyses of agro-morphological data across the three locations were conducted following restricted maximum likelihood (REML) and mixed model procedures (Hardy and Thompson, 1996; Payne and Senn, 2007), considering locations as fixed effects and accessions as random effects to calculate the genotypic variance (σ^2_g), genotype × environment interaction variance ($\sigma^2_{g\times e}$) and the error variance (σ^2_ε) of the experiments. Grain mineral nutrient contents of the 225 accessions evaluated at the Wad Medani site were used to estimate entry means, genotypic variance (σ^2_g) and residual variance (σ^2_ε) following the REML analysis (Hardy and Thompson, 1996), considering the genotypes as random effects. Best linear unbiased predictors (BLUPs) were also estimated. Entry mean-based heritabilities of all agro-morphological traits were estimated as

$$H^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_{g\times e}/l + \sigma^2_\varepsilon/rl),$$

while the plot-based repeatabilities for the nutritional traits were calculated as

$$h^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_\varepsilon/r),$$

where r is the number of replications per environment and l is the number of environments (Hallauer and Miranda, 1988; Singh and Ceccarelli, 1995; Piepho and Möhring, 2007).

Pearson's coefficients of correlation were calculated among the grain mineral nutrient contents as well as between the grain mineral nutrient contents and agro-morphological traits for the Wad Medani data to determine the associations among the nutrients studied and also their relationships with the other morphological traits. Box plots were used to illustrate the extent of agro-morphological diversity among the 20 accessions with highest contents of grain mineral nutrients such as Fe, Zn, Ca and Na.

Principal component analysis was computed using adjusted entry means and a biplot was obtained to illustrate the relationships among the 15 agro-morphological and nutritional traits for the Wad Medani data. The biplot was generated by plotting the entries according to their scores of the first and second principal components. For a grouping of genetic materials according to their morphological similarities, a cluster analysis was performed using adjusted entry means for the 15 agro-morphological and nutritional traits across the three sites, centred to mean zero and scaled to unit variance, following an agglomerative hierarchical clustering algorithm (Ward, 1963).

Results

Agro-morphological diversity

Entry means across the three locations showed mostly a wide range and significant ($P < 0.001$) genetic variation for the agro-morphological traits studied (Table 1 and Table S1, available online). Estimates of genotype ×

Table 1. Means, minimum and maximum values, variance components with standard errors (SE) for genotypes (σ^2_g) and genotype × environment interactions ($\sigma^2_{g\times e}$), and estimated entry mean-based heritabilities (H^2) for agro-morphological traits of the 225 pearl millet accessions evaluated in 2010 at three sites in Sudan

				Variance components		
Traits	Mean	Min	Max	$\sigma^2_g \pm SE$	$\sigma^2_{g\times e} \pm SE$	H^2
Plant height (cm)	151.6	118.5	187.6	88.5 ± 9.7**	79.2 ± 1.1**	0.81
Productive tillers/plant	3.1	2.0	4.6	0.2 ± 0.3**	0.1 ± 0.5ns	0.72
Plant population/m^{2}[a]	4.1	1.3	6.3	0.13 ± 0.3**	0.4 ± 0.6*	0.49
Days to 50% flowering	69.1	62.2	77.7	4.9 ± 1.4**	2.2 ± 1.9**	0.80
Panicle length (cm)	23.1	16.2	34.4	6.3 ± 1.2**	1.1 ± 1.7**	0.85
Panicle density (score, 9 best)	5.4	2.0	8.5	1.2 ± 0.6**	0.6 ± 0.8**	0.83
1000-seed weight (g) [a]	9.0	7.9	10.1	0.3 ± 0.4**	4.1 ± 0.4**	0.27
Grain yield (g/m^2)	173.0	79.6	267.8	418.3 ± 37.1**	392.7 ± 11.7**	0.89
Dry forage yield (g/m^2)	510.3	296.6	739.8	2442.2 ± 76.3**	1861.1 ± 125.0**	0.74
Harvest index (%)	24.1	13.5	39.4	13.4 ± 3.2**	26.3 ± 3.5**	0.59

ns, Non-significant.
*, ** Significant at $P < 0.05$ and $P < 0.01$, respectively.
[a] Variance components multiplied by 10.

environment interaction variance were also highly significant ($P < 0.001$) for agro-morphological traits except for number of productive tillers, panicle exsertion and synchrony of panicle maturity, but mostly smaller than the genotypic variance, which resulted in high heritability estimates for most of the investigated traits (Table 1).

Variability in grain mineral nutrient contents

Genetic differences among the accessions were also highly significant for all the ten grain mineral nutrient contents and β-carotene concentrations (Table 2 and Table S1, available online). The concentration of β-carotene was very low for most of the accessions; however, some accessions such as HSD_2129, HSD_2178, HSD_2191 and HSD_7138 showed relatively high β-carotene levels of 7.0, 7.0, 7.2 and 8.2 μg/100 g, respectively (Table S1, available online).

Frequency distributions

Most of the agro-morphological traits investigated were normally distributed (Fig. S1, available online). Of the accessions, 68% showed a plant height of 140 to 170 cm, while 18% revealed a short plant height of less than 130 cm. Also, 50% of the accessions had a panicle length ranging from 25 to 34 cm, while 13% showed shorter panicle lengths. The majority of the accessions (75%) flowered between 67 and 72 d, while only nine accessions required more than 76 d to reach 50% flowering.

Table 2. Means and ranges (minimum and maximum values), variance components with standard errors (SE) for genotypes (σ^2_g) and estimated plot-based repeatabilities (h^2) for grain nutritional traits of the 225 pearl millet accessions evaluated at Wad Medani (Sudan) under irrigation in 2010

Traits	Mean	Min	Max	$\sigma^2_g \pm SE$	h^2
Fe (mg/kg)	42.9	19.7	86.4	1.3 ± 0.2***	0.88
Zn (mg/kg)	40.3	13.5	82.4	1.1 ± 0.3***	0.91
Ca (mg/kg)	229.1	73.5	603.5	44.9 ± 4.9***	0.89
Na (mg/kg)	10.4	3.6	44.5	0.1 ± 0.1***	0.88
Cu (mg/kg)	7.3	1.6	12.4	0.1 ± 0.1***	0.90
Mg (mg/kg)	1371.0	118.6	1933.1	821.2 ± 3.4***	0.86
Mn (mg/kg)	15.9	6.6	39.1	0.3 ± 0.1***	0.89
K (mg/kg)	5463.2	1444.7	8583.5	5847.5 ± 9.6***	0.78
P (mg/kg)	4023.5	626.7	5705.5	4932.4 ± 9.1***	0.87
S (mg/kg)	1643.7	867.5	2237.7	1510.9 ± 4.5***	0.86
β-Carotene (μg/100 g)	3.4	1.0	12.6	0.1 ± 1.0***	0.79

*** Significant at $P < 0.001$.

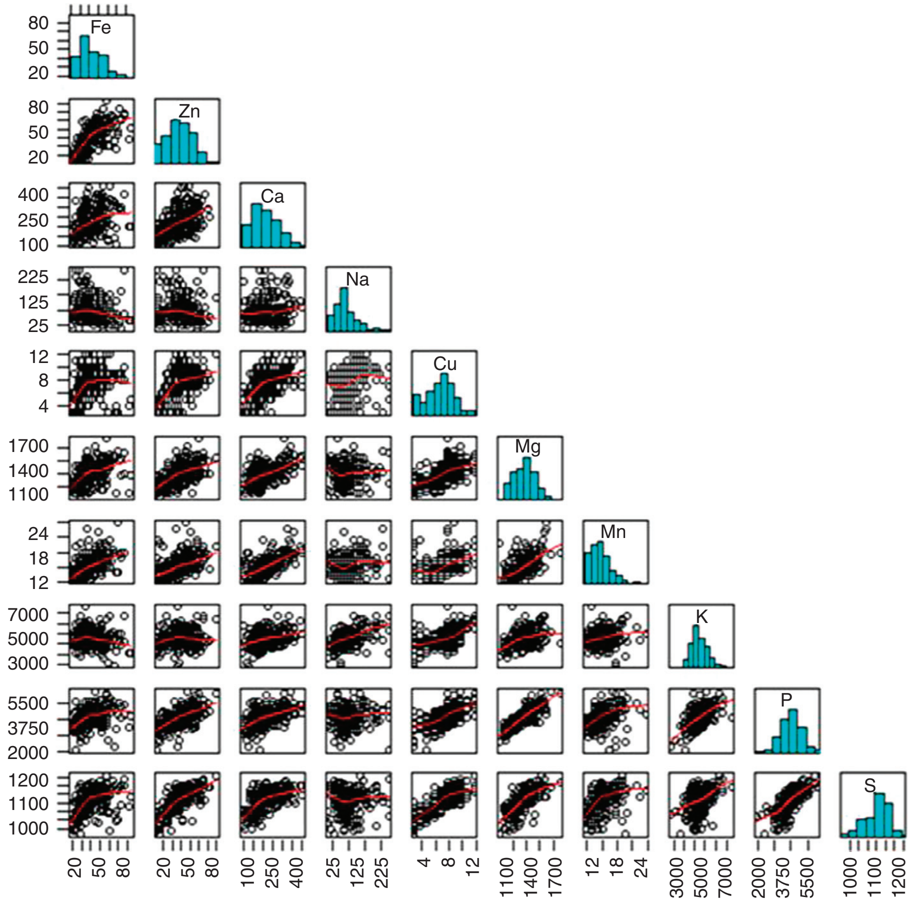

Fig. 1. (colour online). Scatter plots showing frequency distributions of and the relationships among the ten mineral content traits for self-pollinated grain samples from the 225 pearl millet accessions, produced in 2010 under irrigation at Wad Medani (Sudan).

Only three accessions had very compact panicles, while 18 accessions were compact and the majority had semi-compact panicles. Frequency distributions were also near normal for most of the grain nutrient contents (Fig. 1).

Coefficients of correlation

Highly significant ($P \leq 0.01$) and positive correlations were observed between Fe or Zn contents and all the other nutrients, except Na and K contents (Fig. 1). The highest correlation among the minerals was found between Mg and P ($r = 0.81$) followed by Fe and Zn ($r = 0.77$), Zn and S ($r = 0.76$) and Cu and S ($r = 0.74$), while no correlations were observed between Zn and K ($r = 0.02$), Na and Mg ($r = -0.02$) and Na and S ($r = -0.02$) (Fig. 1).

The coefficients of correlation estimated between the mineral nutrient contents and yield traits for the Wad Medani site were very low and mostly not significant (data not shown). Days to flowering and panicle length were negatively correlated with all the investigated nutrients, except for Fe with days to flowering ($r = 0.03$) and Mn with panicle length ($r = 0.03$). Therefore, apart from these exceptions, early flowering and short panicles were associated with higher grain nutrient contents. No relationships were observed between 1000-seed weight and any of the nutrients assessed. Grey-coloured grains had the highest average Fe content, but a wide variation for the Fe content was observed within each grain colour class (Fig. S2, available online).

No substantial association was observed among the agro-morphological traits (data not shown), and only high grain yield performance was moderately related to early flowering ($r = -0.29$), pointing to some limited importance of escape from terminal drought stress.

Principal component 1 (PC1) and 2 (PC2) explained 34 and 13%, respectively, of the total variation (Fig. 2). In the resulting biplot, the 225 pearl millet accessions were distributed throughout the four sections. The biplot illustrates strong positive associations among all the

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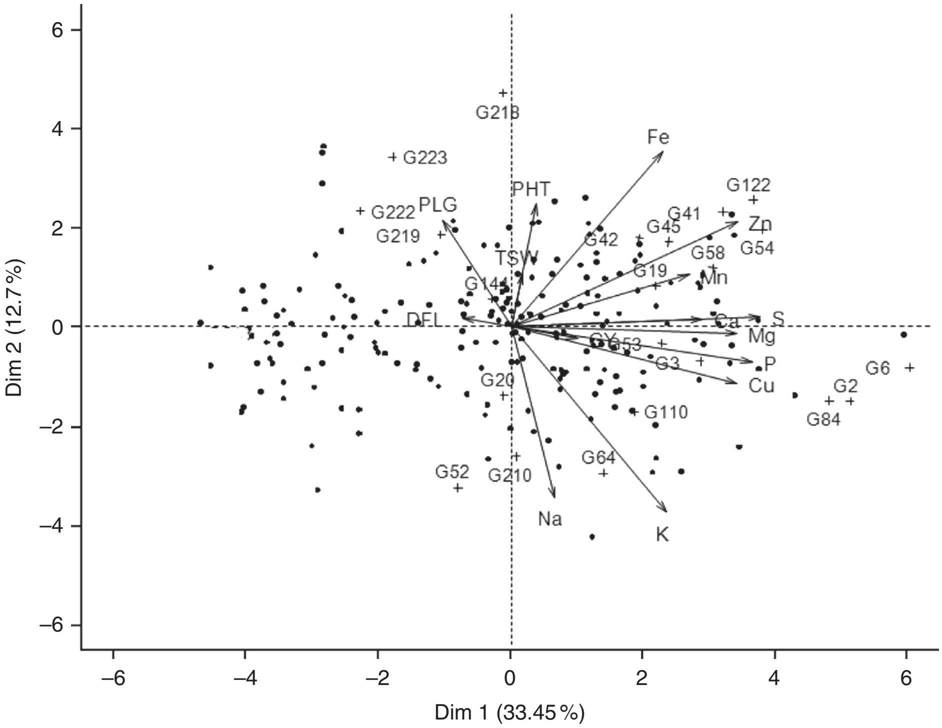

Fig. 2. Principal component biplot of the 225 pearl millet accessions and different nutritional and agro-morphological traits for the Wad Medani site. Vectors indicate the various traits; crosses mark the accessions (G) with highest nutritional values; black circles denote the remaining pearl millet accessions. DFL, days to 50% flowering; PHT, plant height; PLG, panicle length; TSW, 1000-seed weight; GY, grain yield; Fe, Zn, Ca, P, K, Mg, Mn, S, Na and Cu, grain mineral nutrient contents (of self-pollinated grain samples).

nutritional traits, especially between Fe and Zn, Zn and Mn as well as among S, Ca, Mg, P and Cu (Fig. 2). Eleven genotypes (G2, G6, G19, G41, G45, G53, G54, G58, G84, G122 and G212) share the highest values (among the top 20) for two or more of the nutrients (Fe, Zn, Ca and Na; Fig. 2).

Variation among high-nutrient accessions

The 20 accessions with highest grain Fe, Zn, Ca and Na contents revealed wide variation for days to 50% flowering, grain yield, 1000-seed weight, panicle length, plant height and panicle density (Fig. 3). On the other hand, no significant differences between the mean values of the agro-morphological traits such as days to 50% flowering, plant height, panicle length, panicle density and grain yield were observed when compared across the four selected sets (Fig. 3), indicating that there was no effect of high grain nutrient contents on the agro-morphological traits.

Geographic differentiation

No clear patterns of geographic differentiation were identified for days to 50% flowering, panicle length, and Fe and Zn concentrations within Sudan (Fig. 4(a)–(d)). All the four investigated traits did not show any correlation with latitude or longitude. However, partial concentration of longer panicles was observed in Darfur and southern Kordofan areas (Fig. 4(b)), and some accessions with high Fe and Zn contents seemed to be concentrated in Darfur and southern Kordofan, which are mountainous areas (Fig. 4(c) and (d)).

Cluster analysis

Cluster analysis differentiated the 225 pearl millet accessions into seven groups based on the BLUPs for the investigated agro-morphological traits (Fig. S3, available online). Accessions with relatively long panicles belonged to group III, while accessions with compact panicles were found in groups I and V. Early-flowering accessions were related to group VII, while later-flowering accessions were observed in groups I and III. Groups I, II, III and IV included accessions with high Fe and Zn contents; the average values of these groups for Fe and Zn contents were greater than the overall mean of all the accessions studied for these traits (data not shown).

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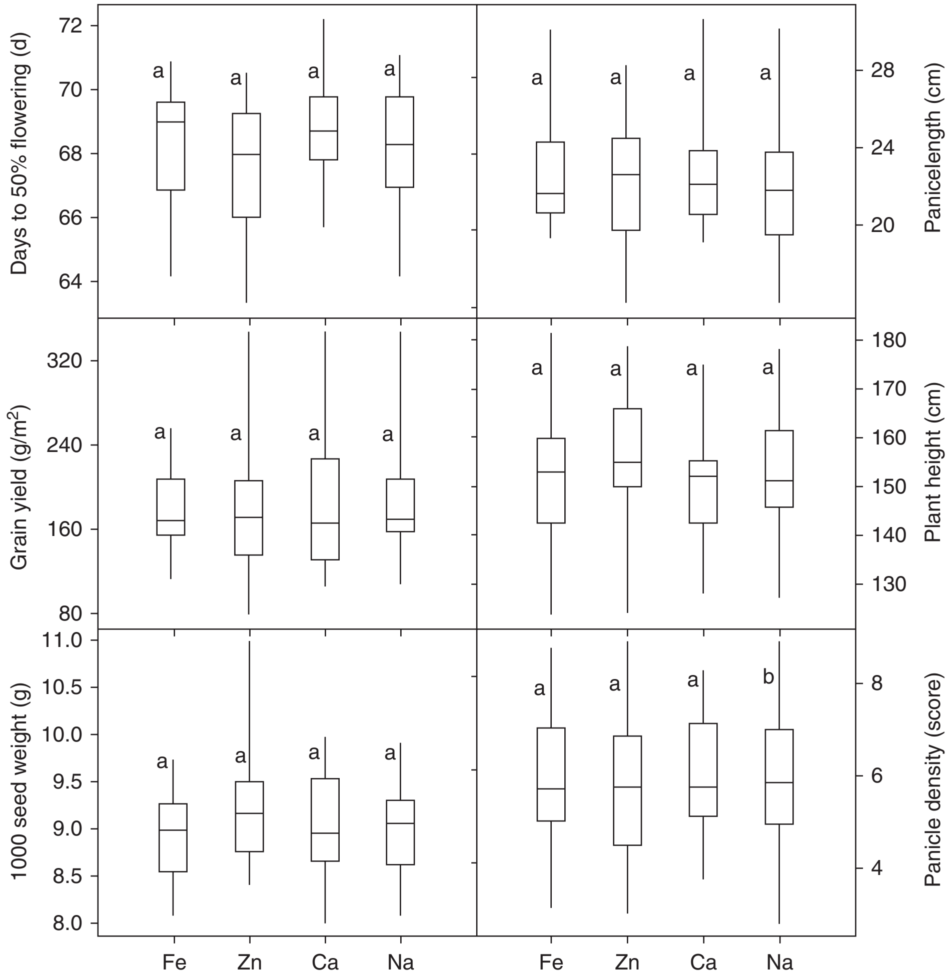

Fig. 3. Box plots of the selected agro-morphological traits of the 20 pearl millet accessions with highest contents of grain mineral nutrients such as Fe, Zn, Ca and Na, respectively. Only trait means within plots marked by different letters (a, b) are significantly different at $P < 0.05$.

Discussion

Appropriate characterization of plant genetic resources for agro-morphological and grain nutrient traits is necessary to facilitate the utilization of germplasm by breeders in crop improvement as well as for genetic resource management. Use of genetic resources in crop improvement is one of the most sustainable methods to conserve valuable genetic resources, and to simultaneously increase agricultural production and food security (Haussmann *et al.*, 2004). Landraces can be the storehouses of valuable genetic variability and are often well adapted to the local soil type, climatic conditions, etc. (Khairwal *et al.*, 2007). Despite the importance of pearl millet in Sudan, not much improvement was achieved with regard to yield and yield stability, and, to our knowledge, no studies have reported on grain nutrient concentrations of Sudanese pearl millet landraces. Therefore, to characterize and enable utilization of pearl millet genetic resources from Sudan, a collection of 225 mainly Sudanese landraces and breeders' lines was examined in this study.

Extent of pearl millet agro-morphological diversity in Sudan compared with other studies

Although there were significant variations and high heritabilities among the Sudanese accessions for most of the agro-morphological traits studied (Table 2), the variation for some traits was limited compared with West African materials, which revealed wider ranges of variation for traits such as flowering time, plant height and panicle length (Haussmann *et al.*, 2006). On the other hand, the extent of variability observed in this study was similar to that reported for Indian pearl millet germplasm from Rajasthan (Sharma *et al.*, 2003). The wide range of diversity for West African materials might be due to the fact that the West African region includes the centre of origin of pearl millet (Harlan, 1971; Tostain, 1992; Oumar *et al.*, 2008; Manning *et al.*, 2011; Clotault *et al.*, 2012). The limited variation observed for some agro-morphological traits in this study means that diversification could be beneficial. Since Sudan is a

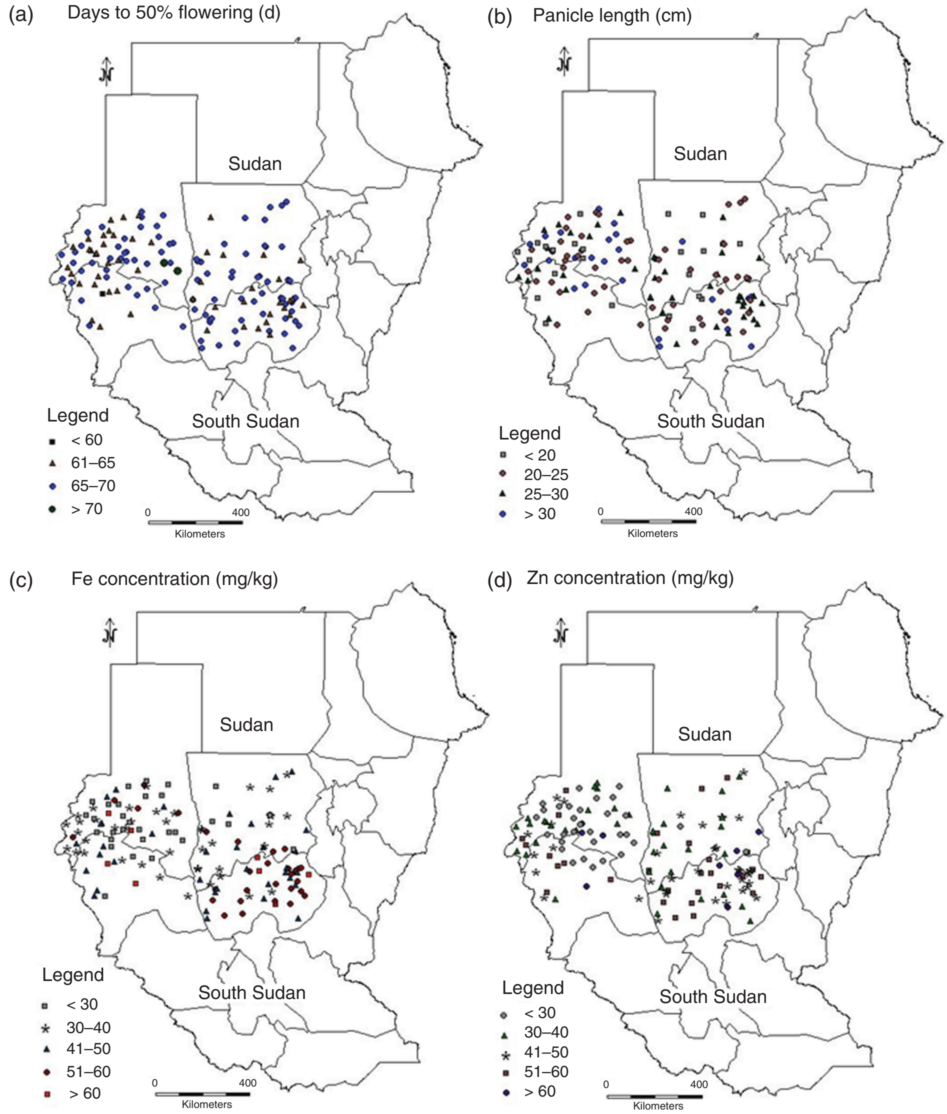

Fig. 4. (colour online). Geographic differentiation of Sudanese pearl millet landraces (145 geo-referenced accessions) for (a) days to 50% flowering, (b) panicle length, (c) self-pollinated grain Fe concentration and (d) self-pollinated grain Zn concentration.

continuation of the West African Sahelian belt, diversification of Sudanese breeding materials can be improved using diverse West African pearl millets, and this could be a promising approach to enhance gains from selection.

Variability of Sudanese pearl millets for grain nutrient content compared with other studies

The concentrations of minerals in grains are influenced by numerous complex, dynamic and interacting factors, including genotype, soil properties, environmental conditions and nutrient interactions (House, 1999). In the present study, differences among the genotypes in absorbing different minerals from the soil and accumulating them in the grains to achieve nutritional benefits were clearly expressed, but since data were obtained from only one site, and from self-pollinated (instead of sibbed) panicles, further investigations regarding the stability of grain nutrient contents are required. The ranges in grain nutrient concentrations of the studied minerals detected in this study were considerably wider than those reported in several studies (Velu *et al.*, 2008a, b; Buerkert *et al.*, 2001). While there was a significant variance observed among the accessions for β-carotene levels, the overall concentration values observed in this study were markedly low compared with those reported by Khangura *et al.* (1980) for pearl millet from India, and relatively high compared with what was found for West African pearl millet (Buerkert *et al.*, 2001). The low concentrations of β-carotene observed in this study may not be enough to cover the daily need of vitamin A for humans. Therefore, germplasm from India with

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high β-carotene concentrations could be used to improve the Sudanese pearl millet. Several Quantitative trait loci (QTL) and candidate genes with significant effects on carotenoid contents have been identified in sorghum (*Sorghum bicolor* (L.) Moench; Fernandez *et al.*, 2008) and maize (*Zea mays* L.; Harjes *et al.*, 2008; Vallabhaneni *et al.*, 2009; Yan *et al.*, 2010); these could also be a starting point for breeding high provitamin A-containing pearl millet. However, according to C. Tom Hash (personal communication), even with markedly higher β-carotene levels, comparable with those of the Indian high β-carotene yellow-endosperm breeding lines reported by Khangura *et al.* (1980), the amount of β-carotene that could be provided from pearl millet grain-based foods would not be nearly enough to meet the recommended daily intake of vitamin A for humans. Furthermore, the β-carotene content of a small-grained cereal such as pearl millet is likely to have a short shelf-life due to oxidation. Finally, there are much better dietary sources of vitamin A, such as moringa (*Moringa* spp.) leaves and yellow-fleshed storage pumpkins (*Cucurbita* spp.), that can be grown in pearl millet production areas and are being consumed in pearl millet production areas in West and Central Africa, and that could be introduced to the relevant areas of Sudan if they are not already known there. Therefore, pearl millet breeding efforts would probably be better focused on other target traits such as pest and disease resistance for which such simple alternatives are not available. In the case of Fe and Zn, dietary studies among pearl millet consumers in India have suggested that pearl millet-based foods are a major source of these mineral micronutrients, so the suggestion for breeding for their enhancement appears to be reasonable, provided that the variation reported can be validated using more robust sampling methods (C. T. Hash, personal communication).

The remarkable genetic variance for grain mineral nutrient concentrations (Table 2 and Table S1, available online) in the accessions studied suggests ample scope for selection of nutrient-rich accessions, and such accessions could be released for cultivation if found agronomically acceptable after extensive evaluation to fulfil the requirements of the target smallholder farmers. Moreover, germplasm with high grain nutrient contents could also be hybridized with agronomically superior accessions/breeding lines to combine high grain nutrient content with farmer and consumer preference traits. When compared with other field crops, the pearl millet materials assessed had comparably higher grain mineral nutrient contents, especially Fe and Zn concentrations, than all other crops (Table S3, available online). This is in agreement with previous studies of these grain mineral micronutrient traits in pearl millet (Velu *et al.*, 2007; Govindaraj *et al.*, 2013), including those based on more representative grain samples than those used in the present study. This finding suggests that these Sudanese pearl millet accessions provide very interesting sources for traits for pearl millet biofortification breeding programmes, provided that the elevated levels detected in this study are validated with more robust sampling methods using sibbed panicles and larger grain samples.

The high repeatability estimates for grain nutritional traits in this study indicated that genotype played an important role in determining the concentration of the nutrients. However, environmental and genotype × environment interaction effects on grain nutrient contents still need to be studied in order to obtain unbiased mean and heritability estimates. Recently, Govindaraj *et al.* (2011, 2013) reported similar high heritability estimates and suggested the predominance of additive gene effects in the inheritance of nutritional traits in pearl millet. The result is also congruent with the conclusion of Graham *et al.* (1999), who reported the existence of significant and sufficient genetic variance and workable heritabilities for the improvement of mineral nutrient concentrations in field crops.

The non-significant differences in the contents of grain mineral nutrients such as Fe, Zn, Ca and Na for the agro-morphological traits between the groups of the 20 best accessions (Fig. 3) suggested that the four nutrients investigated had no greater influence on the selected agro-morphological traits. However, it is known that seeds rich in minerals such as Zn can give agronomic advantages to the crop such as high seedling vigour, high level of disease resistance as well as high water-use efficiency of the crop (Cakmak *et al.*, 1999). Such traits are of importance especially in the harsh environments of sub-Saharan Africa. The wide range of variation observed within the four nutrient-rich groups for flowering time, grain yield, plant height and panicle length suggests a great opportunity for the development of biofortified pearl millet with different phenology and agro-morphological traits that may be suited to different environments. In addition, the highly nutritious accessions that showed high grain yield can be distributed to subsistence farmers after validating farmer preferences.

Exploitation of trait relations in pearl millet improvement

The strong positive relationships among the grain nutritional traits reflected that either the genetic factors or the physiological functions for the uptake/translocation in the grain of these nutritional traits are interconnected. These results are in line with Jiang *et al.* (2007) who found close correlations among the mineral nutrient contents in rice. Generally, it can be concluded that breeding

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activities aiming to enhance the concentration of some nutrients in pearl millet grain can also lead to the improvement of other related nutrients. Such co-response of related nutrients enhances the chance to develop pearl millet varieties rich in several mineral nutrients.

The lack of associations among the nutritional and agro-morphological traits means that both types of traits can be easily combined, and there are no forced directions for each type when selecting for the other. However, many previous reports have shown that grain yield was negatively associated with some of the mineral nutrient contents (Jiang *et al.*, 2007; Selvi and Rajarathinam, 2009; Šimić *et al.*, 2009). Moreover, Prasad (2010) also mentioned that the concentration of nutrients generally declines as the yield increases, which could simply be a dilution effect. On the other hand, Ashok Kumar *et al.* (2009), Brkić *et al.* (2004) and Murphy *et al.* (2008) disagreed with that. The existence of high-yielding accessions with moderately high levels of certain mineral nutrients in the present study confirms the possibility of simultaneous improvement for both types of traits in pearl millet – assuming that the variation in seed set under the selfing bags did not contribute significantly to the observed variation in grain mineral micronutrient content values obtained from the self-pollinated grain samples used for grain mineral content analysis. Moreover, the wide ranges of Fe contents detected in each grain colour class suggested that Fe-rich pearl millet can be developed regardless of the grain colour and, therefore, farmers and consumers can select according to their preferences.

The relationships illustrated in the biplot (Fig. 2) describe a negative association between panicle length and Zn content, and this result is in line with the findings of Jiang *et al.* (2007). In the present study, early-flowering accessions tended to have a higher grain yield (perhaps due to the escape from terminal drought stress) with high Fe and Zn contents in their grains; these trait correlations should facilitate the selection of nutrient-rich and high-yielding varieties in Sudan. The biplot generally illustrated the good prospects for developing nutrient-rich pearl millet cultivars with a wide range of agro-morphological characteristics and therefore different adaptation potential, contributing to the alleviation of poverty and malnutrition problems in poor communities. Furthermore, the biplot gives an opportunity to select contrasting genotypes of pearl millet with different minerals or agro-morphological traits to be introduced in further hybridization programmes.

Geographic differentiation of pearl millet in Sudan

Information obtained on the patterns of geographic differentiation for days to flowering and panicle length showed that distribution of these traits throughout the study region of Sudan depends mainly on farmer preferences or other environmental factors such as soil type. In francophone West Africa, the geographic differentiation of pearl millet landraces revealed much clearer geographic patterns especially for days to flowering, with early-flowering materials being prevalent in the north and late-flowering landraces being prevalent in the south (Haussmann *et al.*, 2006). This corresponds to the rainfall gradient in the region. Furthermore, some geographic differentiation for panicle length was observed in West Africa, with long-panicle types being dominant in Niger and Senegal (Haussmann *et al.*, 2006).

Prospects for using the observed genetic diversity in pearl millet breeding

The distribution of accessions from different geographic origins across different cluster groups indicated poor evidence for the association between the geographical origins of the accessions and the patterns of the clusters. However, the cluster analysis in this study was based on agro-morphological traits; high-density molecular markers may be required to derive conclusions about the genetic differentiation of Sudanese pearl millets in relation to geographic distance. Thete *et al.* (1986) and Shanmuganathan *et al.* (2006) also found no association between the patterns of clusters and the geographic distribution of pearl millet accessions in India. Wilson *et al.* (1990) reported that the distribution of pearl millet landrace collection sites within major clusters tended to support the premise that the landraces are gene pools differentiated in adaptation to various regions of Burkina Faso.

Our study identified distinct clusters of accessions representing complementary traits. These may form different heterotic groups. Crosses among members of different clusters might be promising for improving pearl millet varieties. However, for more validation, distinct clusters should be established based on genetic diversity as assessed by molecular markers that are linked to performance traits, and systematic evaluation of putative intra- and interpool crosses to assess combining ability patterns in the germplasm. Building the breeding programme on the concept of heterotic groups could result in productivity gains and enhanced yield stability not only in pearl millet hybrids, but also in open-pollinated populations or synthetic cultivars that may serve smallholder farmers better in the short term.

Conclusions

The studied collection of more than 200 Sudanese pearl millet accessions contains significant genetic variation for agro-morphological traits, but the improvement of Sudanese breeding materials by using appropriate West African germplasm could further enhance useful genetic variance and therefore gains from selection. The large variation observed for grain nutrient contents revealed the potential usefulness of this germplasm for pearl millet biofortification programmes. This variation should now be validated using more representative grain sampling methods using sibbed panicles on at least a subset of the accessions. Due to positive correlations among different grain nutrients and the lack of correlations between grain nutrients and agro-morphological traits, it is possible to develop high-yielding varieties that are rich in several micronutrients. These could be valuable contributions to reducing malnutrition and hidden hunger in Sudan and other rural pearl millet-growing areas of the world.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262113000233

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