Agro-morphological Characterization of West and Central African Pearl Millet Accessions

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ABSTRACT

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] was domesticated in Sahelian West Africa. This highly outcrossing crop is one of the most important staple cereals in the semiarid tropics, adapted to very harsh rain-fed conditions. Agro-morphological characterization of local germplasm is very important to better understand existing diversity, ease targeted genetic broadening of breeding populations, and potentially link this knowledge to genotypic information. The objectives of our study were to (i) characterize West and Central African (WCA) pearl millet accessions based on their agro-morphological traits; (ii) evaluate the possibility to group accessions based on their agro-morphological characteristics; (iii) determine geographic patterns of phenotypic differentiation; and (iv) derive conclusions for pearl millet improvement in WCA. A total of 360 early-to-medium maturity accessions were phenotyped for 12 agro-morphological traits at six environments in WCA. Wide ranges of all observed traits indicated a high diversity of the tested accessions. Principal component analysis revealed very large diversity within individual countries, especially within Mali and Burkina Faso. Some limited grouping of accessions from Niger, Senegal, Cameroon, Morocco, and Mauritania was observed for individual principal component axes. Geographical differentiation and country differences were detected for several traits. The results and data presented in our study reflect WCA pearl millets' tremendous diversity and adaptability to a wide range of environments and give a sound basis for breeders to select and utilize this germplasm to serve the manifold needs of WCA pearl millet farmers.

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Abbreviations: G × E, genotype by environment; ICRISAT, International Crops Research Institute for the Semi-Arid Tropics; IER, Institute of Rural Economy; INERA, Institute of the Environment and Agricultural Research; INRAN, National Agricultural Research Institute of Niger; IRD, Institut de la Recherche pour le Développement; ISRA, Senegalese Institute for Agricultural Research; LCRI, Lake Chad Research Institute; OPV, open-pollinated variety; PCA, principal component analysis; WCA, West and Central Africa.

P EARL MILLET 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 (Haussmann et al., 2012). 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.

The earliest evidence of domesticated pearl millet has been found in the Lower Tilmesi Valley in northeastern Mali, where the cereal was cultivated already 4500 yr ago (Manning et al., 2011). As it is hypothesized that pearl millet was first domesticated in West Africa (Oumar et al., 2008), the region is considered a diversity hotspot for this cereal, so exploitation of its germplasm should be very valuable for plant breeding. Pearl millet, is a predominantly cross-pollinated species with an outcrossing rate of more than 75% (Sandmeier, 1993), and robust desiccation-tolerant pollen (Hanna, 1990; Hoekstra et al., 2006) can effect gene flow across great distances.

Even today, WCA farmers growing this cereal predominantly use pearl millet landraces (Busso et al., 2000; Dingkuhn et al., 2006). The long history of individual selection, with continuous gene flow between domesticated and wild types, has ensured the maintenance of pearl millet diversity (Bezançon et al., 2009; Lewis, 2010). Therefore, characterization of germplasm from this diversity hotspot and its overall adaptation patterns in the surrounding target region will be crucial to efficient use of this diversity in breeding.

Until recently, the use of improved open-pollinated varieties (OPV) of pearl millet in WCA was very limited due to low availability of seed and insufficient infrastructure in the seed sector, as well as relatively low adoption by the farmers, as improved varieties often did not suit their preferences (Ndjeunga, 1997; Smale et al., 2012). However, in the last decade, a number of initiatives have been undertaken to strengthen farmer involvement in pearl millet improvement, such as on-farm variety testing and certified seed production, which have resulted in increasing farmers' access to improved pearl millet cultivars (Omanya et al., 2007). Seed systems are also being strengthened in the region (Toenniessen et al., 2008).

National pearl millet breeding programs in WCA continue to focus mainly on their national collections and hesitate to introduce external material, even from countries that share the same agro-ecology and that might therefore have similarly adapted germplasm pools. The present agro-morphological characterization of a diverse panel of landraces was undertaken to encourage local breeders to broaden their local breeding materials to reach higher gains from selection.

In addition to characterization of accessions, the assessment of population structure and diversity patterns is a relevant aspect for breeding programs that develop open-pollinated varieties, synthetics, or hybrids. In particular, hybrid breeding requires information on the genetic distinctiveness of accessions to develop heterotic groups, which are characterized by their wide genetic distance from each other and excellent combining abilities toward the opposite group, resulting in maximal hybrid performance (Melchinger and Gumber, 1998). Pearl millet hybrid breeding is just being initiated in WCA; it is thus very important to support this process and identify concomitant heterotic grouping with agro-morphological and genetic diversity studies.

The objectives of the present study were (i) to characterize 360 WCA pearl millet accessions for agro-morphological and performance traits in 6 environments; (ii) to evaluate the possibility to group accessions based on their phenotype; (iii) to identify geographic patterns for specific traits; and (iv) to derive conclusions for pearl millet improvement in WCA.

MATERIALS AND METHODS
Plant Material and Phenotypic Evaluation

The study was based on 360 early-to-medium maturing accessions, representing a wide geographic origin out of 16 WCA countries (Supplemental Table S1). Of these accessions, 347 were landraces, and the remaining 13 were improved, OPVs. The landraces had been collected jointly by Institut de la Recherche pour le Développement (IRD; formerly ORSTOM, Office des Recherches Scientifiques et Techniques d'Outre-Mer) and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) during collection missions in 1976 and 2003, and were obtained from IRD-Montpellier and IRD-Niamey. Unfortunately, pearl millet diversity from Nigeria was underrepresented in this collection.

All 360 accessions were grown under rain-fed conditions in 2006 at six sites in WCA: ICRISAT Sahelian Center research station Sadoré, Niger; National Agricultural Research Institute of Niger (INRAN) research station Maradi, Niger; Senegalese Institute for Agricultural Research (ISRA) research station Bambey, Senegal; Institute of Rural Economy (IER) research station, Cinzana, Mali; Lake Chad Research Institute (LCRI) research station Maiduguri, Nigeria; and Institute of the Environment and Agricultural Research (INERA) research station Gampela, Burkina Faso (Table 1).

The design of the experiment was an α-lattice with three replicates and eight entries per incomplete block in each environment. One experimental unit consisted of two rows with a length of 4.8 m, a distance of 0.75 m between rows, and 0.8 m between hills within a row (seven hills per row). Thinning was done to two plants per hill. All trials were fertilized using the micro-dosing technique (6 g NPK–15–15–15/hill, corresponding to 93 kg ha^{-1}) applied at planting, followed by a urea topdressing of 50 kg ha^{-1} after thinning. At the Maradi site only (following local recommendations), an additional 100 kg ha^{-1} of super simple phosphate were applied before planting. Manual weeding was done as required.

Phenotypic traits evaluated on plot basis were seedling vigor (rating from 1 to 5; 1 = worst, 5 = best), flowering time (days to 50% flowering after sowing, with flowering meaning full stigma emergence on main panicle), percentage of downy mildew-infested plants, plant height (in cm), panicle length (in cm), panicle circumference (in cm), number of productive tillers per hill, grain yield (in g m^{-2}), dry stover yield (in g m^{-2}), thousand kernel weight (in g), number of grains per panicle (calculated using grain yield per plot, number of productive

Table 1. Information on experimental sites.

| Site | Latitude | Longitude | Altitude (m) | Soil type | Average rainfall[†] (mm) | Rain in 2006 (May–October) | | Date of planting (all in 2006) |
						Total (mm)	N rainy days	
Bambey	14.7091	-16.4793	20	Sandy	611.4	478.7	39	25/07
Cinzana	13.2537	-5.9682	282	Sandy loam	630.1	758.9	58	24/07
Gampela	12.4192	-1.3505	275	Sandy loam	814.7	751.8	51	22/07
Sadoré	13.2375	2.2797	235	Sandy	556.2	561.4	40	14/07
Maradi	13.4895	7.0925	380	Sandy	560.2	513.7	49	21/07
Maiduguri	11.8345	13.1523	320	Sandy	620.6	599.7	45	24/06

[†]Source of average rainfall: www.worldclimate.com/ (accessed 19 Jan. 2015).

panicles per plot, and thousand kernel weight), compactness of panicle (rating from 1 to 3; 1 = not compact, 3 = very compact), and volumetric weight of grains (in g L^{-1}). The latter trait is of farmers' interest, since the market prices for pearl millet depend on volumetric units. Plant height, panicle length and panicle circumference were measured on three representative plants per plot, and the average taken forward for analysis.

Due to operational problems, flowering time, downy mildew susceptibility, thousand kernel weight, and volumetric weight of grains were not recorded in Maradi, and seedling vigor was not recorded in Maradi and Bambey.

Statistical Analysis

For each trait, the adjusted entry means across the six environments were calculated by a one-step approach using a mixed model, where heterogeneous error variance of environments was assumed. The following model was used:

$$y_{ijklm} = \mu + g_i + e_j + ge_{ij} + r_{jk} + b_{jkl} + \varepsilon_{jklm}$$

where y_{ijklm} represents the observed phenotype on plot basis; μ, the overall experimental mean; g_i, the genetic effect of genotype i; e_j, the effect of environment j; ge_{ij}, the interaction between genotype i and environment j; r_{jk} the effect of replication k nested in environment j; b_{jkl}, the effect of incomplete block l nested in replication k and environment j; and ε_{jklm}, the residual effect of the plot m nested in block l, replication k, and environment j. For calculation of adjusted entry means, genotypic effects were treated as fixed and all the other factors as random, while for estimation of variance components genotypic effects were treated as random as well. Significance of variance components was tested by model comparison using the likelihood ratio test (Stram and Lee, 1994). Broad sense heritability estimates were calculated as:

$$h^2 = \sigma_g^2 \times (\sigma_g^2 + \frac{\sigma_{ge}^2}{E} + \frac{\sigma_\varepsilon^2}{ER})^{-1}$$

being the genotypic variance; σ_{ge}^2, the genotype by environment (G × E) variance, and σ_ε^2, the residual error variance for R replicates and E environments. The 95% confidence intervals of h^2 were calculated according to Knapp and Bridges (1987).

The principal component analysis (PCA) was based on centered adjusted means across environments of all above-mentioned traits except grain yield.

The Euclidian distances among accessions were based on the same data as the basic PCA and were used to determine the correlation between morphological distance and geographic distance between accessions.

The Pearson coefficients of correlation were used to determine the relationships among traits and between traits and latitude and longitude of the origin of the genotypes. To calculate the correlation for downy mildew, three landraces (PE08407 and PE08408 from Morocco and PE08065 from Mauritania) were excluded, because their adjusted means for downy mildew susceptibility were exceedingly high and would have biased the results. The significance levels of the correlation coefficients were Bonferroni-adjusted for the number of simultaneous tests.

Box plots were based on the adjusted entry means of accessions, which were grouped by their country of origin, while only the four countries with the highest number of accessions and traits with a heritability above 0.5 were considered.

All calculations were performed within the R-environment v. 2.14.2 (R Development Core Team, 2012). Mixed model analyses were performed using the package ASReml v. 3.0 for the R-environment (Butler et al., 2009), and maps were plotted using the software DIVA-GIS 7 (version 7.4.0, Hijmans et al., 2012).

RESULTS

Differentiation for Agro-morphological Traits

Wide ranges were observed for all traits, based on the adjusted means for each accession across six environments (Table 2). For example, plant height varied from 129 cm to 293 cm, panicle length from 17 cm to 89 cm, circumference from 6 cm to 11 cm, and downy mildew susceptibility from 2% to 83%. The accessions, which had previously (during their actual collection) been classified as early-to-medium maturing, showed a range of 52 d in time to 50% flowering.

Estimates of σ_g and $\sigma_{g\times e}$ were highly significant ($P < 0.01$) for all traits (Table 2). Downy mildew susceptibility was the only trait that showed a very high magnitude of G × E interaction, with a σ_{gxe}/σ_g ratio of about 6, causing a low heritability ($h^2 = 0.30$). In comparison to this low heritability across locations, repeatabilities for the same trait determined for each environment were rather high ($w > 0.72$, Supplemental Table S1). Reflecting the high G × E interaction, phenotypic correlations among environments for downy mildew susceptibility were rather low

Table 2. Means, ranges and variance components with standard errors (SE) for genotypes (σ_g), genotype-by-environment interaction (σ_{gxe}), and heritabilities (h^2) for various traits based on 360 pearl millet accessions evaluated in six environments in West Africa.

| Traits[†] | Mean ± SE | Range | Variance components | | h^2 | 95% C.I. on h^2 [‡] |
			σ_g ± SE	σ_{gxe} ± SE		
SV	3.77 ± 0.36	1.98 - 4.53	0.11** ± 0.01	0.05** ± 0.01	0.38	0.27, 0.47
FT (days)	69.89 ± 1.70	49.27 - 101.41	70.03** ± 5.40	5.38** ± 0.43	0.90	0.89, 0.92
DM (%)	17.49 ± 9.30	1.77 - 82.60	28.23** ± 5.67	167.67** ± 8.5	0.30	0.18, 0.41
PH (cm)	227.24 ± 12.56	128.54 - 292.91	466.86** ± 38.06	68.66** ± 8.72	0.71	0.66, 0.75
PL (cm)	39.66 ± 2.33	17.38 - 89.31	182.25** ± 13.87	5.90** ± 0.75	0.91	0.90, 0.93
PC (cm)	8.08 ± 0.66	6.02 - 10.82	0.59** ± 0.05	0.20** ± 0.02	0.65	0.59, 0.70
NPT	3.7 ± 0.50	1.59 - 6.50	0.44** ± 0.04	0.17** ± 0.02	0.41	0.31, 0.50
GY (g m^{-2})	91.4 ± 15.24	-9.41 - 162.20	576.44** ± 51.51	323.68** ± 23.06	0.62	0.55, 0.67
DSY (g m^{-2})	310.81 ± 44.16	76.71 - 681.25	6349.76** ± 556.4	2830.42** ± 226.88	0.59	0.52, 0.65
TKW (g)	8.48 ± 0.62	2.98 - 11.38	1.61** ± 0.13	0.32** ± 0.03	0.75	0.71, 0.79
NGP	2664.68 ± 640.10	292.34 - 5237.08	391109.06** ± 32666.0	60246.8** ± 8205.95	0.47	0.38, 0.55
CP	2.01 ± 0.23	0.82 - 3.03	0.13** ± 0.01	0.04** ± 0.01	0.51	0.42, 0.58
VW (g L^{-1})	809.16 ± 12.70	740.02- 847.15	312.74** ± 26.34	40.20** ± 7.48	0.55	0.47, 0.62

** Significant at the 0.01 probability level.

† Traits are: SV, seedling vigor; FT, days to 50% flowering; DM, downy mildew susceptibility; PH, plant height; PL, panicle length; PC, panicle circumference; NPT, number productive tillers per hill; GY, grain yield; DSY, dry stover yield; TKW, thousand kernel weight; NGP, number of grains per panicle; CP, compactness of panicles; VW volumetric weight.

‡ Confidence intervals of h^2 were calculated by the method of Knapp and Bridges (1987).

and partially even negative (between $r = -0.26$ and $r = 0.21$, Supplemental Table S3). Significantly positive correlations among environments for downy mildew susceptibility were only found between Gampela, Sadoré, and Cinzana ($r > 0.16$, $p < 0.01$). For all other traits, correlations among environments were considerably higher.

Seedling vigor, number of productive tillers per hill, grain yield, and dry stover yield revealed significant G × E interaction effects, with σ_{gxe}/σ_g ratios between 0.3 and 0.6 (Table 2). In general, heritabilities for the observed traits were very variable, with highest values for panicle length ($h^2 = 0.91$) and flowering time ($h^2 = 0.90$). Rather low heritabilites ($h^2 < 0.41$) were observed for seedling vigor, number of productive tillers per hill, and, as mentioned above, downy mildew susceptibility.

Relationships Among Traits

Flowering time was significantly correlated to many of the observed traits (Fig. 1). For instance late flowering was associated with relatively lower downy mildew susceptibility ($r = -0.34$, $p < 0.001$), tall plant height ($r = 0.62$, $p < 0.001$), and high dry stover yield ($r = 0.68$, $p < 0.001$). The longer vegetative growth period of later maturing genotypes therefore increased height and biomass. Early flowering accessions tended to have higher thousand kernel weight ($r = -0.34$, $p < 0.001$) and grain volumetric weight ($r = -0.36$, $p < 0.001$). An increase in plant height was significantly associated with a decrease in downy mildew susceptibility ($r = -0.25$, $p < 0.001$) and an increase in panicle length ($r = 0.42$, $p < 0.001$). Panicle length was also highly significantly correlated to number of grains per panicle ($r = 0.55$, $p < 0.001$).

Seedling vigor was strongly positively correlated to grain yield ($r = 0.63$, $p < 0.001$), indicating the importance of good seedling vigor to achieve a high grain yield. The yield components thousand kernel weight, number productive tillers per hill, and number of grains per panicle were only moderately, but significantly, correlated to grain yield ($r = 0.21$ to 0.35, $p < 0.001$), while panicle circumference and panicle length were not significantly correlated to grain yield.

Correlations among traits observed within each environment separately (Supplemental Table S2), showed variable coefficients in the different environments, indicating interactions between trait correlations and environment. The degree of variation between environments was dependent on the trait. Correlations between flowering time and dry stover yield (ranging from $r = -0.14$ to $r = 0.65$), flowering time and grain yield (ranging from $r = -0.54$ to $r = 0.04$), and thousand kernel weight and compactness of panicle (ranging from $r = -0.38$ to $r = 0.26$) showed particularly high dependence on the environment.

In case of seedling vigor and grain yield, correlations ranged from $r = 0.40$ ($p < 0.01$) in Cinzana to $r = 0.54$ ($p < 0.01$) in Gampela, all of which were below the correlation coefficient determined based on the adjusted means across all environments.

Geographic Differentiation

Agro-morphological traits showed more significant associations with the latitude of the accessions's geographic origin than with the respective longitude. For instance, latitude was significantly negatively correlated with flowering time ($r = 0.27$, $p < 0.001$), indicating later maturity
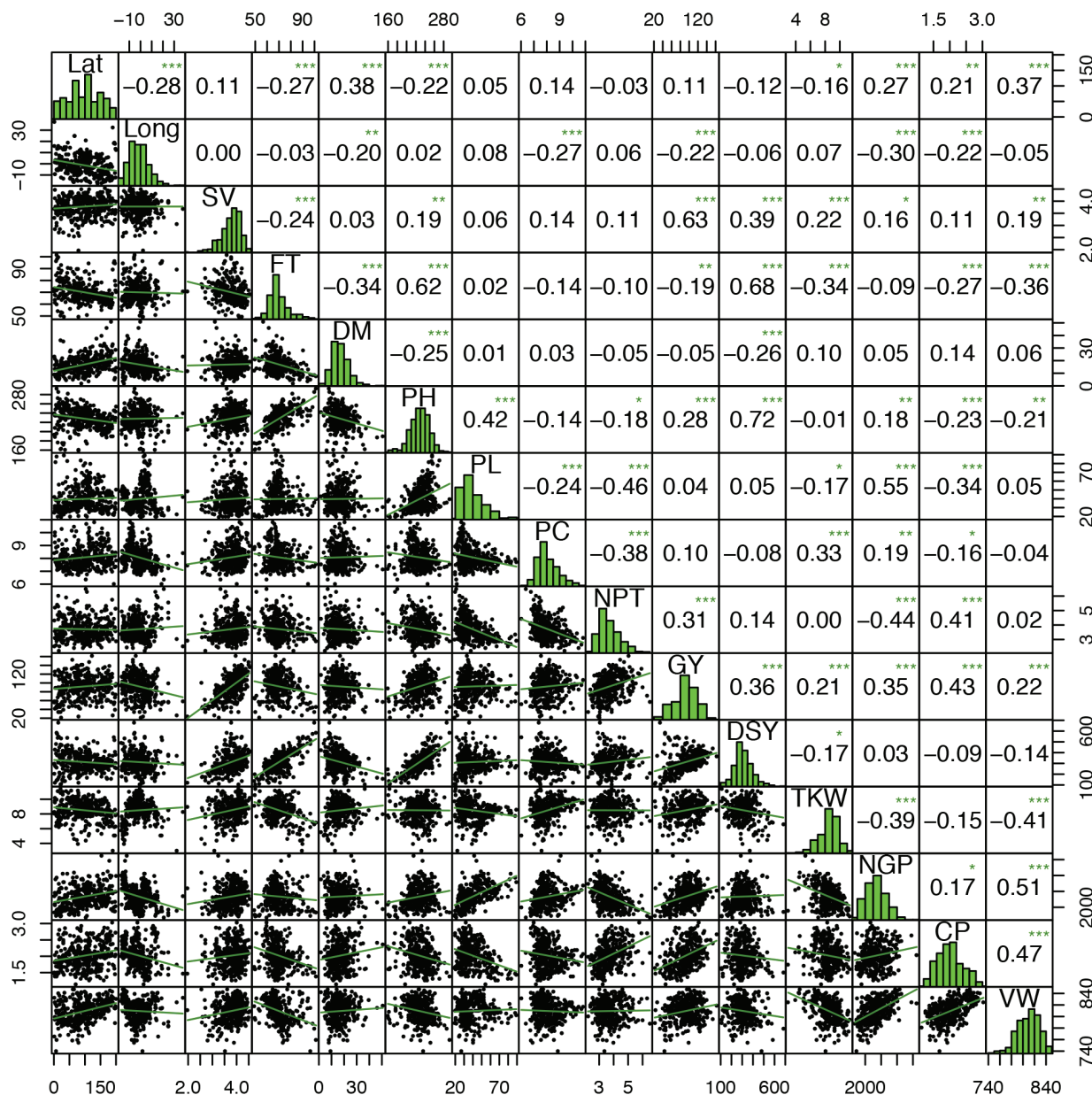
Figure 1. Correlation coefficients (above diagonal) and linear regressions (below diagonal) among latitude (Lat) and longitude (Long) of the genotypes' geographic origin and agro-morphological traits (abbreviations and units, see Table 2), based on 360 pearl millet accessions and their adjusted means across six environments in West Africa. *, **, *** Significant at 0.05, 0.01, and 0.001 probability levels after Bonferroni-adjustment, respectively.

of accessions from the south (Fig. 1), while flowering time was not correlated with longitude.

Downy mildew susceptibility was significantly positively correlated with latitude of the accessions's geographic origin ($r = 0.39$, $p < 0.001$), while it was significantly negatively correlated with the respective longitude ($r = -0.20$, $p < 0.01$), indicating that accessions from northern Senegal and Mauritania tend to be generally more susceptible than accessions from Benin or Nigeria. Negative correlation between longitude and panicle circumference ($r = -0.27$, $p < 0.001$) and number of grains per panicle ($r = -0.30$, $p < 0.001$) hint that accessions from the western part of

WCA have larger panicle circumference and larger number of grains per panicle than those from the eastern part.

Among the observed traits, mean flowering time and panicle length of the 360 accessions averaged across six environments were chosen to illustrate geographic differentiation (Fig. 2). In the case of flowering time, there is a gradient of earlier to later flowering from the north to the south, i.e., from lower to higher mean annual precipitation (Fig. 2A). For example, central Mali and southern Mauritania, which receive less than 350 mm rainfall, originate accessions with flowering time below 67 d, whereas southern Mali and southern Burkina Faso, with more than 800 mm rainfall, originate accessions with flowering time
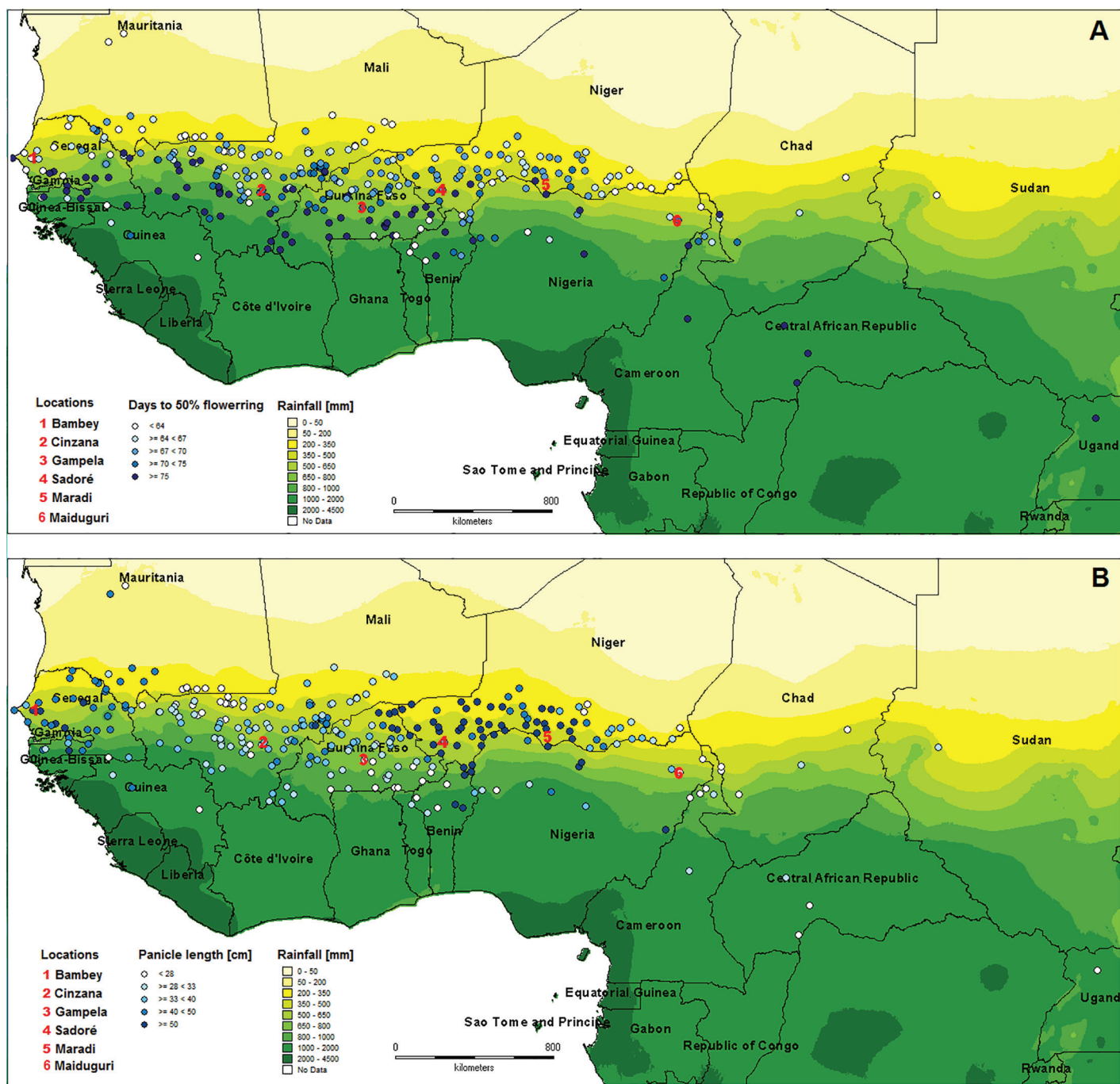
Figure 2. Origin of 360 accessions (circles), environment of test sites (red numbers), and geographic differentiation for days to 50% flowering (A) and panicle length (B) (color codes).

greater than 75 d. However, it can be noted that some extra-early varieties are also cultivated in the southern parts of WCA, e.g., in the border area of Burkina Faso with Ghana, Togo, and Benin.

The geographic distribution of panicle length indicates a regional clustering of this trait (Fig. 2B). For instance, panicles of Senegalese and Nigerien accessions are relatively long (mostly > 40 cm), whereas accessions from Mali and Burkina Faso have predominantly shorter panicles (mostly < 40 cm). A gradient of panicle length along longitude or latitude is not visible on the map,

which is also supported by the nonsignificant correlations between panicle length and longitude or latitude.

Comparison of Pearl Millet Diversity Within Countries

The PCA based on 12 traits (excluding yield) revealed that the first four principal components explained 23.6%, 18.6%, 16.9%, and 13.4% of the phenotypic variation, respectively (Fig. 3). Accessions from Mali and Burkina Faso were widely scattered, which indicates that the diversity within each of those countries covers a high degree of
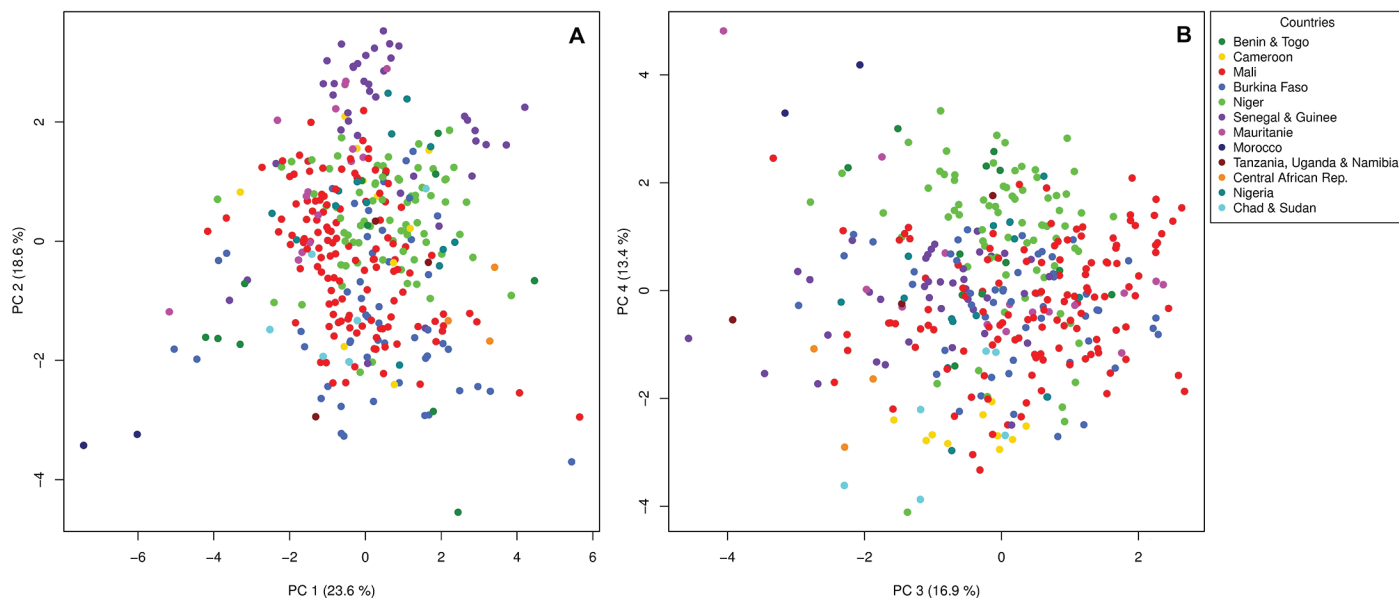
Figure 3. Principal component analysis [PC1 vs. PC2 (A) and PC3 vs. PC4 (B)] of 360 West African pearl millet accessions based on 12 agro-morphological traits evaluated in six environments. Countries of origin of accessions are indicated by color.

the overall phenotypic diversity present in the 360 accessions. Accessions from Niger are clustered closer to each other than those from Mali and Burkina Faso. In Fig. 3A, most accessions from Senegal were clustered relatively close to each other (PC1 and PC2 values above -1 and 0, respectively) and were slightly separated from the others, while no grouping was observed in Fig. 3B. The two accessions from Morocco were separated from the majority of accessions in both plots. Except one, accessions from Mauritania were clustered relatively close to each other in Fig. 3A, but not in B. For Cameroon, close clustering was observed in Fig. 3B, but not in Fig. 3A.

Box plots, where accessions were grouped by their country of origin, showed that the mean and variation of several traits varied with country (Fig. 4). In particular, panicle length, thousand kernel weight, compactness of panicle, and volumetric weight showed large differences in mean values between countries. The boxplots for panicle length illustrated that accessions from Niger had, on average, the longest panicles and at the same time the greatest variation. Accessions from Senegal had generally lower thousand kernel weight, higher compactness of panicle, and higher volumetric weight than accessions from the other three countries. Accessions from Senegal had, on average, the largest number of grains per panicle, followed by Niger. Accessions from Burkina Faso had, on average, the lowest number of grains per panicle (data not shown).

DISCUSSION
Extent of Diversity in WCA Pearl Millet
The highly significant genetic variation and the wide ranges for several traits observed in this collection of 360 WCA pearl millets underlines the very impressive pearl

millet diversity that exists in West Africa, even within a limited, predominantly early-to-medium maturity group. This is not so surprising, as West Africa is one of the centers of origin for pearl millet (Oumar et al., 2008; Manning et al., 2011). The observed agro-morphological diversity also reflects pearl millet's adaptability to a wide range of contrasting environments and the diversity of farmer preferences in this region. From this it can be concluded that there will never be a one-size-fits-all pearl millet cultivar, not even for geographically limited areas within the same agro-ecological zone. This diversity needs to be tapped and used efficiently in pearl millet improvement to match varietal options to specific contexts and to serve well the diversity of needs of smallholder farmers.

The multi-environment characterization data summarized in this paper and made publicly accessible in the supplemental files is expected to facilitate more targeted use of this diversity in pearl millet improvement programs in WCA and worldwide. The data may also be useful for specific genetic studies of this important food security crop.

Because the tested pearl millet materials belong to the early-to-medium maturity group, their photoperiodic response is generally not very strong (Clerget et al., 2007; Haussmann et al., 2007). The fact, that the mean number of days to 50% flowering was not significantly different at the different locations despite variable planting dates (Supplemental Table S1) supports this suggestion. However, because the present study's planting dates are confounded with latitude and other location effects such as rainfall distribution, no clear conclusions can be drawn regarding the effect of planting date on the expression of flowering time and other traits.
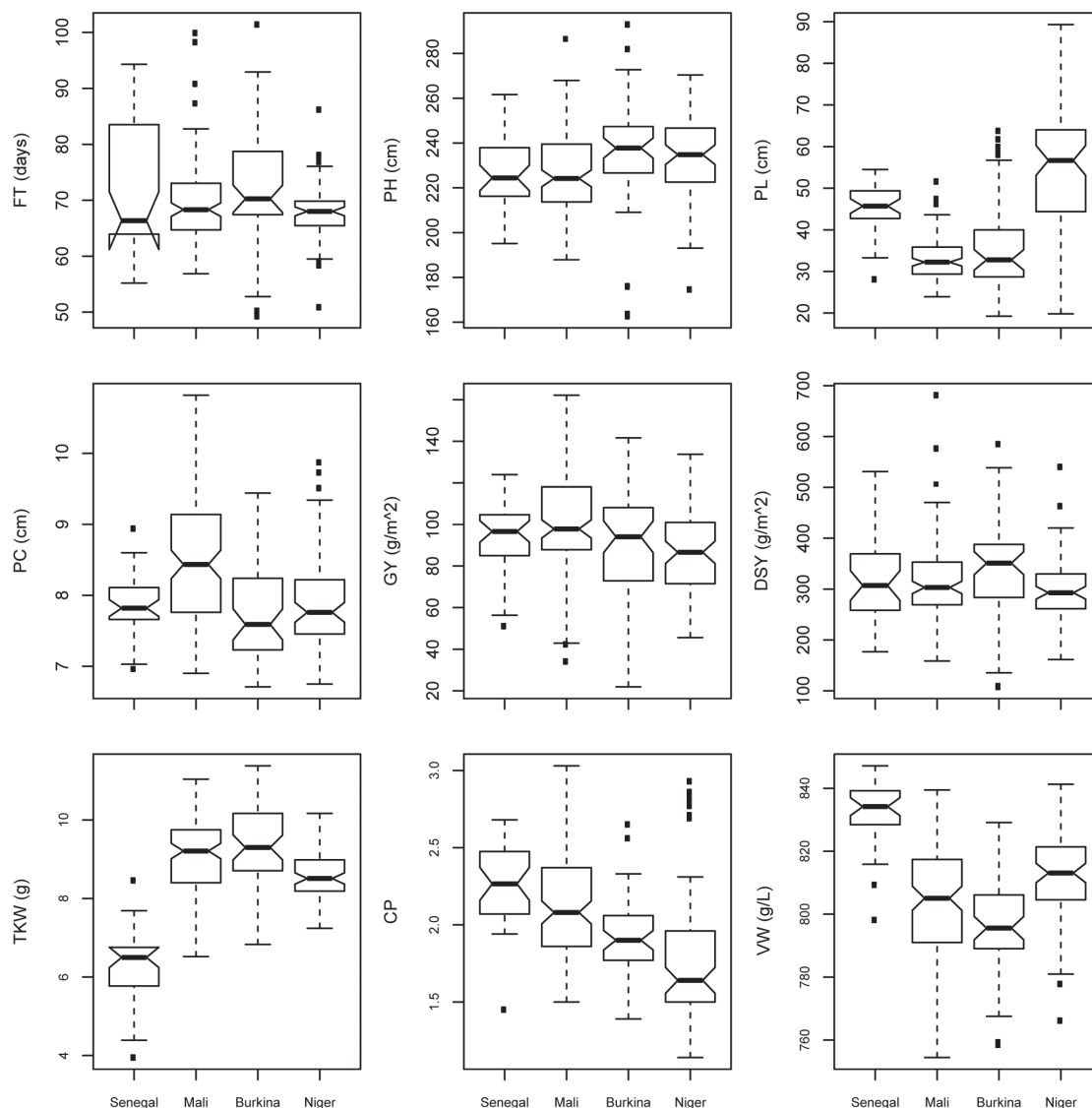
Figure 4. Box plots of nine traits, based on adjusted means of genotypes across all six test environments, grouped by their country of origin. Number of genotypes per box plot: Senegal = 36, Mali = 118, Burkina Faso = 53, Niger = 75 (abbreviations, see Table 2).

The ranges of agro-morphological traits observed in our study are slightly lower relative to what Upadhyaya et al. (2011) and Bhattacharjee et al. (2007) reported for the phenotypic diversity of a global (ICRISAT) pearl millet collection when characterizing 21,594 and 21,392 accessions, respectively. However, a direct comparison of our data with the diversity found by these authors is not possible for several reasons. The two studies showed the diversity of accessions from all over the world, while our accessions were limited to West African origin with early-to-medium maturity. Furthermore, the global collection was tested in only one environment in India, whereas our accessions were tested in six environments in West Africa, which shrinks the adjusted entry means and ranges toward the overall mean.

Meaningful Trait Correlations

Seedling vigor was significantly and positively correlated with grain yield at all individual locations ($r = 0.40$ to r

$= 0.54$, Supplemental Table S2) and across sites ($r = 0.63$, Fig. 1). The observed correlations underline the importance of good early seedling establishment across the whole range of test environments, which is in line with the study of Manga and Yadav (1995). Particularly on low P soils, which are a major constraint in the target region, good early seed establishment has considerable advantage (White and Veneklaas, 2012).

The correlation between flowering time and grain yield varied across test environments. At Bambey and Sadoré, and to a limited extent also at Maiduguri, early-flowering genotypes tended to yield higher, which was probably caused by terminal drought stress limiting grain yield performance of later-flowering accessions. This assumption can be supported by Bidinger et al. (1987), who found a strong negative correlation between flowering time and grain yield when pearl millet receives terminal drought stress. Selection potential for both grain yield and biomass

seems limited in this type of environment because of the significant association of late flowering with high biomass. At Gampela and Cinzana, flowering time was not related to grain yield, and some later-flowering accessions had high grain yield in these environments. Because of the high interannual rainfall variability common in West Africa (Haussmann et al., 2012), the relationship between grain yield and flowering time can be expected to vary not only across sites, but also across years at individual sites. This indicates that selection for a specific, uniform flowering time (e.g., determined as "best" in one test year or even across test years) may not be the best strategy. It does not seem to be by chance that local pearl millet landraces in West Africa maintain variability for flowering time (Haussmann et al., 2007), as this seems to be a good strategy to cope with interannual rainfall variability and concomitant variable relationships between flowering time and grain yield (Haussmann et al., 2012).

The finding of a negative correlation between plant height and downy mildew susceptibility might be justified by a negative effect of the disease on plant height, which has been assessed by Nahunnaro et al. (2013). A more probable explanation is that early flowering time is strongly associated with short plant height ($r = 0.62$), and the negative correlation of downy mildew susceptibility with flowering time ($r = -0.34$) largely carries through to plant height as well ($r = -0.25$).

Compactness of panicles and grain volumetric weight were positively correlated ($r = 0.47$), indicating that selection for one of the two traits could improve both, albeit likely at the cost of reduced thousand kernel weight. Considering that compactness of panicle had a higher correlation to grain yield, a focus on the compactness might have a higher relevance and impact than volumetric weight. In fact, panicle compactness is a major trait used by farmers to select superior materials in participatory population improvement (Haffke, unpublished data, 2010).

Genotype-by-Environment Interaction for Downy Mildew Susceptibility and Grain Yield

All six test locations included in the present study are within the range of environments that are expected to be suitable for the target collection of early-to-medium maturity pearl millet accessions. Within that range of environments, downy mildew susceptibility showed a very high magnitude of G × E interaction compared to the other traits (Table 2), which is likely to be explained by the matching of pathogen population virulence frequency with resistance alleles present in host populations present in a particular environment (both pathogen and host are highly allogamous populations in nature) and accession-specific resistance to particular pathogen populations. This underlines the importance of pathogen variability for virulence and site-specific selection for pearl millet downy mildew resistance across the

test locations. Those results are in line with the study by Kountche et al. (2013), who also found very high G × E interaction for downy mildew susceptibility in pearl millet field trials conducted in Niger and Mali, and QTL mapping studies that have shown different genomic locations for resistances effective against pathogen isolates from geographically distant collection sites (Jones et al., 1995; Breese et al., 2000; Hash and Witcombe, 2001).

The very low correlations among environments for downy mildew susceptibility support the generally low comparability between the downy mildew pathogen populations present in those environments and the assertion that accessions resistant at one location may not necessarily be resistant at other locations. Moderate positive correlations between Gampela, Sadoré, and Cinzana ($r > 0.16$, $p < 0.01$, Supplemental Table S3) indicate that those three environments are most similar regarding genotypic ranking for downy mildew resistance/susceptibility and that results obtained in one location might be, to some extent, transferable to the other locations that are positively correlated. But these correlations are not strong enough to permit selection for resistance against a single pathogen population to provide resistance that is effective across a wide range of such pathogen populations.

Grain yield showed relatively high G × E interaction as well, indicating that different accessions were best performing in different environments. In our study, pair-wise correlations between the six environments ranged from $r = 0.35$ (Bambey and Cinzana) to $r = 0.59$ (Gampela and Maiduguri). Best correlations were found among Gampela, Maiduguri, and Maradi ($r > 0.55$), indicating that those three environments ranked the accessions in similar ways in the test year (2006) and could therefore be considered as a mega-environment. While this grouping of the Gampela, Maiduguri, and Maradi environments in 2006 is also very visible in the AMMI biplot (Supplemental Fig. 1), this finding needs to be validated across several years, due to the extremely high interannual rainfall variability commonly observed in WCA (Haussmann et al., 2012). The AMMI biplot also illustrates that Sadoré and Bambey ranked accessions in similar ways, which could be explained by the common occurrence of terminal drought stress in those two environments in 2006, leading to superior performance of earlier flowering genotypes in both sites (see trait correlations in Supplemental Table S2).

Geographic Distribution

Several associations between agro-morphological traits and latitude seem to be explained by the increase in precipitation and the rainy season duration in southern WCA. For instance, the adaption to shorter rainy seasons leads to early maturing genotypes, which is in line with several other studies (Stich et al., 2010; Vigouroux, Mariac et al., 2011). The higher downy mildew susceptibility resistance of southern accessions might be explained by the

adaptation to higher downy mildew susceptibility pressure caused by higher precipitation and humidity there.

The mean and variation of traits within four countries demonstrated by the box plots (Fig. 4), showed a significant influence of the countries of origin. Such information could be interesting, for instance, for a Senegalese breeder, as it demonstrates that Senegalese accessions have in general a lower thousand kernel weight but higher volumetric weight than accessions from other countries. Hence, it seems that an increase in thousand kernel weight in the Senegalese varieties would be easily achieved by introducing germplasm from countries such as Mali or Burkina Faso. However, before changing yield components like thousand kernel weight, the farmer preferences in target regions should be ascertained.

Geographic distances between accessions and phenotypic distance between accessions showed a low significant correlation ($r = 0.18$, $p < 0.001$). Therefore, a wide geographic distance does not imply a high phenotypic difference between accessions. This is in line with a study by Mariac et al. (2006), who found a low correlation ($r = 0.11$, $p < 0.001$) between geographic and genetic distance determined by microsatellite markers for a large sample of cultivated pearl millet accessions collected from Niger.

One explanation for this finding might be a high impact of farmers' selection, which is specific to their preferences and related to the number and type of varieties one farmer grows. For example in Niger, the majority of farmers grow at least two different, complementary varieties: one earlier-flowering, often shorter-panicle variety and one later-maturing, often longer-panicle variety. Chantereau et al. (2010) reported that, on average, six different varieties are grown in a single village in Niger, corresponding to different farmers' preferences. Because of careful seed management and a certain degree of isolation by flowering time, this diversity is being maintained within villages (Bezançon et al., 2009).

The fact that most pearl millet farmers in WCA usually grow at least one earlier- and one later-flowering cultivar is also responsible for the observed correlation between flowering time and latitude ($r = -0.29$), which appears to be rather low, considering the inherent north-south gradient for total amount of rainfall and, correspondingly, the gradient for length of the rainy season observed in WCA. However, the map of flowering time (Fig. 2A) clearly shows that early varieties are being cultivated even in the more southern parts of WCA, while late-flowering varieties cannot be found in northern WCA. The early varieties grown in the more southern areas are cultivated to provide food grain during the "hungry period," when grain stores are empty and the later-maturing millets are still growing in the field.

That farmers' preferences can largely shape geographic patterns of diversity becomes visible in the geographic distribution of panicle length (Fig. 2B). This clearly shows that farmers in Niger and Senegal prefer long panicles, while in Burkina Faso and Mali farmers select shorter panicles. In Niger, long panicles are considered advantageous when it comes to transport, because long panicles can very easily be bound into bundles ("bottes") and then transported on the owners' heads from the field to the grain stores. In Mali and Burkina Faso, where shorter-panicle pearl millet types are grown, the habit of transporting harvested panicles in such head bundles is less common.

Usefulness of the Present Data for Heterotic Grouping

The yield potential of pearl millet is substantially dependent on the heterozygosity of the genotypes, which can be influenced by breeding open-pollinated, synthetic, and hybrid varieties. Several studies on Indian and West African pearl millet have shown superiority of hybrid varieties compared with population varieties (Burton and Powell, 1968; Bidinger et al., 1994; Ouendeba et al., 1995; Yadav et al., 2000). In hybrid breeding, development of heterotic groups is a basic requirement for efficient breeding of highly heterozygous, well-adapted hybrid varieties. Phenotypic and/or genotypic diversity studies are therefore the first step to determining potential heterotic groups. Based on our PCA analysis, only little evidence of geographically distinct groups of accessions could be identified. Even when some clustering was observed for one country based on PC1 vs. PC2, this was generally not confirmed by PC3 vs. PC4, or vice versa. This finding is similar to the results given by Stich et al. (2010) and Lewis (2010), who studied the diversity of 145 WCA inbred lines and 66 WCA pearl millet populations, respectively. The PCAs of both studies were based on genotypic data and, as in our study, did not show distinct groups. Additionally, Lewis (2010) found no geographical structure in the genetic differentiation of pearl millet, which is in line with our study.

Considering that pearl millet was domesticated in Mali (and perhaps elsewhere across today's Saharan, Sahelian, Sudanian, and Guinean ecological zones of WCA) — and from there spread across today's pearl millet cropping areas in Africa, Asia, and the Americas, with continuous and extensive gene flow between the cultivar and its wild and weedy relatives in WCA, as well as between neighboring cultivated varieties as a result of their shared protogynous flowering habit and robust wind-borne pollen — it is not surprising that we find one meta-population and no distinct grouping of accessions. Although there are no distinct groups, one could identify genotypes that are phenotypically relatively distant and complementary to each other, and validate the combining ability of such accessions to determine their potential to be used as heterotic groups in hybrid breeding.

Role of Pearl Millet Diversity in Adaptation to Variable and Changing Climates

Beside growing different varieties, intravarietal diversity can be very beneficial in the harsh and variable cropping environment, because heterogeneous varieties can buffer the risk of yield losses within the population (Haussmann et al., 2012). Although this was not tested in this study, we can assume that pearl millet accessions have a high intravarietal diversity due to the high out-crossing rates of the species (Mariac et al., 2006).

Climate change is an important aspect for which detailed examination of the diversity and agro-morphological characterization could be very utile (Bezançon et al., 2009). An increase in temperature and frequency of drought periods will induce farmers in affected regions to shift their farming systems (Vigouroux, Barnaud et al., 2011). Changes in crops or breeding better adapted varieties could be long-term approaches to adapt to the new climate conditions. A study by Eyshi Rezaei et al. (2013) showed that substituting maize with pearl millet would be a very promising way to increase fodder production in the drought-prone regions of Iran. If the pearl millet growing area will expand through such crop changes, new pearl millet breeding programs have to be established that develop locally adapted varieties. The data given in the supplement could be a good source of information from which to identify germplasm with favorable or desired agro-morphological traits.

In Situ Conservation of Pearl Millet Diversity in West Africa

Conservation of West African pearl millet diversity in its major center of origin and diversity is crucial to ensure that germplasm exhibiting desirable alleles will be available for future breeding programs.

A study by Bezançon et al. (2009) showed that farmers' management of pearl millet cultivars did preserve genetic diversity of pearl millet over nearly 30 yr; no signs of varietal erosion were found between 1976 and 2003 in Niger. In situ conservation by farmers is thus a very important and viable complement to ex situ conservation in gene banks. As farmers manage diversity to reduce cropping risks associated with unpredictably variable rainfall and the generally very harsh environmental conditions that are prevalent in the West African Sahel, pearl millet breeding programs in this region should support and encourage farmers' cultivation of diversity. An excellent way would be to use characterized diversity in breeding programs to develop improved versions (including genetically heterogeneous hybrid varieties) for the different pearl millet types existing in WCA. Moreover, it will be important to continue to offer pearl millet cultivar diversity to the diversity of farmers and to match variety options to farmers' diverse socio-ecological contexts in WCA. Further strengthening of local seed systems that

contribute to maintenance and use of local and improved cultivar diversity also will be important in this regard. The increasingly strong farmer organizations existing in West Africa could play an important role in such local, decentralized seed systems that maintain diversity in situ for the benefit of smallholder pearl millet farmers there (and for future users of pearl millet globally).

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