Pearl millet [Pennisetum glaucum (L.) R. Br.] is able to grow in environments with low precipitation and high temperatures (>42°C; Yadav et al., 2015), where other crops would not survive. In addition, it can cope with low soil fertility such as low soil P levels, which are prevalent in West Africa (WA) (Gemenet et al., 2015a, 2016b). Pearl millet is a staple cereal crop for >90 million smallholder farmers worldwide and possesses good nutritional properties (Sawaya et al., 1984; Varshney et al., 2017). It can provide Fe and Zn (Bashir et al., 2014a), which are deficient in diets of the WA human population. Additionally, the stover is crucial for WA farmers to feed their cattle and build huts.

ABSTRACT

Diets of West African (WA) smallholder farmers are built on pearl millet [Pennisetum glaucum (L.) R. Br.]. Sustainable pearl millet hybrid breeding is challenging in WA, mostly due to an extensive genetic diversity combined with a high degree of admixture. In the absence of natural heterotic groups, understanding combining ability patterns can enable systematic development of heterotic groups and make sustainable hybrid breeding feasible. The objectives of this study were to evaluate heterosis and combining ability patterns and their relationship with genetic distance among WA pearl millets based on population hybrids, and to derive conclusions for future breeding programs. Therefore, 17 open-pollinated varieties (OPVs) were crossed in a diallel mating design and tested together with their offspring in nine environments over 2 yr in Niger and Senegal. Genetic distances between the OPVs were evaluated with twenty microsatellite markers. Average panmictic better-parent heterosis (PBPH) was 18% (1–47%) for panicle yield. A principal coordinate analysis based on genotyping results separated parental OPVs clearly by geographic origin. Although there was no relationship between genetic distance among OPVs and PBPH, we confirmed good combining ability among selected OPVs from Niger vs. Senegal. The identified cultivars (Nigerien CIVT, H80-10Gr, and Taram and Senegalese Thialack 2 and Souna 3) with high combining ability are recommended for founding divergent heterotic pools targeting long-panicle pearl millet hybrids. Our study shows the benefits of population hybrids and represents an important step to identify combining ability patterns and initial heterotic groups for WA pearl millet hybrid breeding.

Identification of Combining Ability Patterns for Pearl Millet Hybrid Breeding in West Africa

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Abbreviations: AMMI, additive main effects and multiplicative interaction; EST, expressed sequence tag; GCA, general combining ability; GxE, genotype × environment; INRAN, Institut National de la Recherche Agronomique du Niger; ISC, ICRISAT Sahelian Center; ISRA, Institut Sénégalais de Recherches Agricoles; MRD, modified Roger’s distance; OPV, open-pollinated variety; PBPH, panmictic better-parent heterosis; PCH, panmictic commercial heterosis; PIC, polymorphism information content; PMPH, panmictic mid-parent heterosis; PC, principal component; PCoA, principal coordinate analysis; SCA, specific combining ability; SSR, simple sequence repeat; WA, West Africa(n).

Pearl millet [Pennisetum glaucum (L.) R. Br.] is able to grow in environments with low precipitation and high temperatures (>42°C; Yadav et al., 2015), where other crops would not survive. In addition, it can cope with low soil fertility such as low soil P levels, which are prevalent in West Africa (WA) (Gemenet et al., 2015a, 2016b). Pearl millet is a staple cereal crop for >90 million smallholder farmers worldwide and possesses good nutritional properties (Sawaya et al., 1984; Varshney et al., 2017). It can provide Fe and Zn (Bashir et al., 2014a), which are deficient in diets of the WA human population. Additionally, the stover is crucial for WA farmers to feed their cattle and build huts.

Published in Crop Sci. 59:1–14 (2019).

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Pearl millet production is still based on landraces and partly open-pollinated varieties (OPVs) (Christinck et al., 2014), which is one of the main reasons for the low grain yields of, on average, 471 kg ha\(^{-1}\) in Niger and 694 kg ha\(^{-1}\) in Senegal (2010–2014). In contrast, Indian farmers started to grow pearl millet hybrid cultivars several decades ago, which, in combination with improved agronomic practices, contributed to an enormous grain yield increase from 305 kg ha\(^{-1}\) (1951–1955) to 1157 kg ha\(^{-1}\) (2010–2014) (Dave, 1986; Yadav and Rai, 2013; FAO, 2017). Hybrid seed production is labor, time, and therefore cost intensive. For example, the Institut d’Economie Rurale in Mali produces small amounts of pearl millet hybrid seeds. They sell these hybrid seeds for CFA2500 (~US$4.30) and OPV seeds for CFA1500 (~ US$2.58) per kilogram (Moussa D. Sanogo, personal communication, 2018). A sufficiently large heterosis effect is necessary to justify the high breeding and seed production costs and, subsequently, much higher seed costs (Duvick and Cassman, 1999). Large heterosis effects can be achieved by crossing genotypes from two genetically distinct heterotic groups. Melchinger and Gumber (1998) defined heterotic groups as groups of genotypes that have a similar combining ability and heterotic response when crossed with a genetically distinct set of genotypes. Furthermore, they emphasized the importance of identifying and maintaining these groups for successful and sustainable hybrid development. Involving common parents in both female and male parental pools may cause a loss of genetic distance, as observed in pearl millet breeding in India by Gupta et al. (2015).

Pearl millet is diploid with 2\(n = 2x = 14\) chromosomes. It is allogamous and protogynous, with drought-tolerant windborne pollen, which leads to outcrossing rates of >70% (Burton, 1974). Moreover, its center of origin is WA (Manning et al., 2011). Therefore, a vast diversity for numerous agromorphological traits can be found in this region (Gemenet et al., 2014; Pucher et al., 2015; Gemenet et al., 2016a; Sattler et al., 2017). In an attempt to identify heterotic groups, Diack et al. (2017) was able to distinguish early- from late-flowering genotypes in Senegal using simple sequence repeat (SSR) markers, but not their combining ability and heterosis. Five overlapping subgroups were identified by Stich et al. (2010) in a set of 145 WA inbred lines using SSR markers. However, without intercrossing the inbreds, it was not possible to observe the relationship between genetic distance between these lines and yield of derived crosses. Likewise, Gemenet et al. (2015b) used diversity array technology (DArT) markers to identify three subgroups in a set of 155 WA pearl millet inbred lines, without describing relations between subgrouping and combining ability. Ouendeba et al. (1993) made first conclusions regarding combining ability by intercrossing five African pearl millet populations (Iniari from Togo, Mansori from Sudan, Ex-Bornu from Nigeria, Ugandi from Uganda, and P\(_3\) Kollo from Niger) in a diallel design. They found large heterosis and significant general combining ability (GCA) effects for several traits, including grain yield. The highest yielding hybrid was derived from crossing Ex-Bornu and P\(_3\) Kollo, which had the two largest GCA effects. Pucher et al. (2016) intercrossed 10 female with 10 male populations, two each being from one of five WA countries, in a factorial mating scheme. In this study, geographic distance was used as a measure for genetic distance between parental populations. The highest yielding hybrids were obtained when crossing Nigerien or Nigerian cultivars with Senegalese cultivars, but overall, there was no significant correlation between geographic distance and heterosis. So far, the high degree of admixture in WA pearl millet germplasm made it impossible to find heterotic groups. Creating groups based on evaluation and augmentation of combining ability between groups of genotypes seems to be more fruitful (Melchinger, 1999). Therefore, large-scale combining ability studies based on factorial or diallel mating designs, which are tested in multi-location and multiyear field trials, are necessary.

Pearl millet hybrid breeding in India is based on single crosses, using parental inbred lines. This approach is difficult in WA due to three reasons. First, the production of pure lines requires more resources, training, and a viable seed industry (Kumara Charyulu et al., 2014). These requirements and a working cytoplasmic male sterility system are available in India (Serba et al., 2017), but not in WA. Second, a significant genetic load is still present in WA pearl millets, due to its outcrossing nature (with heterozygosity masking recessive deleterious alleles) and the fact that WA landraces have not been inbred before. Therefore, inbreds are often highly susceptible to abiotic and biotic stresses and have a low seed set, making hybrid seed too expensive. Third, uniform single-cross hybrids are more vulnerable to stresses like drought and downy mildew caused by *Sclerospora graminicola* (Sacc.) J. Schroet, which bears a tremendous risk for WA subsistence farmers (van Oosterom et al., 1996; Weltzien et al., 1998; Hausmann et al., 2012). Hausmann et al. (2012) highlighted the importance of dynamic (“individual buffering”) and static (“population buffering”) stability as defined by Becker and Leon (1988). These mechanisms can be found in genetically heterogeneous OPVs and will support yield stability, which is crucial in highly variable environments of WA. Cultivars that are easier to develop and have higher yield stability than single-cross hybrids but do exploit heterosis to a larger extent than OPVs will help to tackle the abovementioned issues. Population hybrids, topcross hybrids (with one parent being a population), or three- or four-way hybrids with parents from two distinct populations are appropriate approaches and have already proven their superiority over OPVs in on station trials (Ouendeba et al., 1993; Pucher et al., 2016).

The objectives of this study were (i) to validate the superiority of pearl millet population hybrids based on crosses between Mauritanian, Nigerian, Nigerien, and
Senegalese cultivars over OPVs in WA; (ii) to evaluate combining ability patterns in WA pearl millets; (iii) to determine genetic relationships between parental populations; and (iv) to derive strategies for hybrid breeding in WA based on quantitative–genetic parameters, genetic relationship, and combining ability patterns.

MATERIALS AND METHODS
Selection of Genetic Material
A total of 60 elite pearl millet populations from WA, 20 each chosen by the ICRISAT Sahelian Center (ISC), the Institut National de la Recherche Agronomique du Niger (INRAN), and the Institut Sénégalais de Recherches Agricoles (ISRA), had been evaluated at three locations (Sadoré and Kollo in Niger, and Bamby in Senegal) in the rainy season of 2014. After these trials, 20 populations were selected, based on their performance (above average) and morphological differences. The majority of the selected cultivars had long panicles and stalks, since these are preferred by farmers in Niger and Senegal. To increase diversity, a few cultivars with short panicles (SoSat C88, Ankoutess) and short stalks (3/4 Ex-Borno) were included. Three cultivars failed to produce enough seed and were discarded (Table 1).

Creation of the Diallel Crosses
To produce the diallel crosses (i.e., to cross the parental populations in all possible combinations), the 17 populations were sown in Sadoré at the ISC research station in the off-season of 2015. After these trials, 20 populations were selected, based on their performance (above average) and morphological differences. The majority of the selected cultivars had long panicles and stalks, since these are preferred by farmers in Niger and Senegal. To increase diversity, a few cultivars with short panicles (SoSat C88, Ankoutess) and short stalks (3/4 Ex-Borno) were included. Three cultivars failed to produce enough seed and were discarded (Table 1).

Table 1. List of open-pollinated varieties used to develop population hybrids, including their country of origin and performance traits like plant height (PHT), panicle length (PLE), and grain yield (GYD) in evaluation trials in three West African environments in 2014.

<table>
<thead>
<tr>
<th>No.</th>
<th>Accession</th>
<th>Country of origin</th>
<th>PHT cm</th>
<th>PLE</th>
<th>GYD g m⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>SoSat C88</td>
<td>Mali</td>
<td>222</td>
<td>28</td>
<td>105</td>
</tr>
<tr>
<td>P2</td>
<td>PE08030</td>
<td>Mauritania</td>
<td>242</td>
<td>56</td>
<td>102</td>
</tr>
<tr>
<td>P3</td>
<td>Ankoutess</td>
<td>Niger</td>
<td>193</td>
<td>26</td>
<td>86</td>
</tr>
<tr>
<td>P4</td>
<td>CIVT</td>
<td>Niger</td>
<td>248</td>
<td>60</td>
<td>97</td>
</tr>
<tr>
<td>P5</td>
<td>H80-10Gr</td>
<td>Niger</td>
<td>245</td>
<td>63</td>
<td>130</td>
</tr>
<tr>
<td>P6</td>
<td>ICVM IS 89305</td>
<td>Niger</td>
<td>258</td>
<td>58</td>
<td>124</td>
</tr>
<tr>
<td>P7</td>
<td>ICVM IS 90311</td>
<td>Niger</td>
<td>226</td>
<td>45</td>
<td>94</td>
</tr>
<tr>
<td>P8</td>
<td>ICVM IS 92222</td>
<td>Niger</td>
<td>248</td>
<td>63</td>
<td>122</td>
</tr>
<tr>
<td>P9</td>
<td>T-18L</td>
<td>Niger</td>
<td>263</td>
<td>64</td>
<td>118</td>
</tr>
<tr>
<td>P10</td>
<td>Taram</td>
<td>Niger</td>
<td>258</td>
<td>66</td>
<td>124</td>
</tr>
<tr>
<td>P11</td>
<td>Zatib</td>
<td>Niger</td>
<td>256</td>
<td>67</td>
<td>127</td>
</tr>
<tr>
<td>P12</td>
<td>3/4 Ex-Borno</td>
<td>Nigeria</td>
<td>138</td>
<td>42</td>
<td>77</td>
</tr>
<tr>
<td>P13</td>
<td>SL117</td>
<td>Senegal</td>
<td>234</td>
<td>53</td>
<td>150</td>
</tr>
<tr>
<td>P14</td>
<td>SL123</td>
<td>Senegal</td>
<td>224</td>
<td>53</td>
<td>154</td>
</tr>
<tr>
<td>P15</td>
<td>SL183</td>
<td>Senegal</td>
<td>222</td>
<td>55</td>
<td>150</td>
</tr>
<tr>
<td>P16</td>
<td>Souna 3</td>
<td>Senegal</td>
<td>236</td>
<td>53</td>
<td>137</td>
</tr>
<tr>
<td>P17</td>
<td>Thialack 2</td>
<td>Senegal</td>
<td>249</td>
<td>66</td>
<td>113</td>
</tr>
<tr>
<td>Discarded</td>
<td>Faringuero</td>
<td>Niger</td>
<td>226</td>
<td>41</td>
<td>68</td>
</tr>
<tr>
<td>Discarded</td>
<td>PE01455</td>
<td>Senegal</td>
<td>245</td>
<td>50</td>
<td>121</td>
</tr>
<tr>
<td>Discarded</td>
<td>PE03012</td>
<td>Senegal</td>
<td>251</td>
<td>53</td>
<td>99</td>
</tr>
</tbody>
</table>

pollen of the respective crossing partner. This ensured that the genetic diversity of each parental population would be represented and enough seed of each hybrid would be produced, aiming for equal amounts of seed in all combinations including reciprocals. In detail, the manual pollination was conducted by (i) covering all emerging panicles with a parchment pollination bag, (ii) collecting pollen-containing bags, (iii) creating a pollen mixture from several individuals of one population, (iv) distributing the pollen mix onto panicles of a different parental population where only the female part flowered and no pollen had been shed yet (protogyny), (v) harvesting the (crossed) seed exclusively from the pollinated “female” panicles, and (vi) bulking the seed of crosses with the same parents regardless of the parent being used as male or female, to exclude possible maternal or paternal effects.

Reciprocal crosses were performed but were bulked afterward. Therefore, this is considered to be a half-diallel mating design including the parents according to Griffing’s Method 2 (Griffing, 1956). This procedure yielded, in most cases, enough seed for two seasons. However, offspring derived from crosses with 3/4 Ex-Borno were not sufficiently available for the rainy season of 2015. Therefore, the off-season of 2016 was used to repeat the diallel crosses, partly to supplement the remnant seed from 2015. This time crosses, with 3/4 Ex-Borno being the only dwarfed cultivar, succeeded.

General Trial Conditions
The population hybrids and their parental populations were tested in the rainy seasons of 2015 and 2016 in a total of nine environments in Niger and Senegal. In the rainy season of 2015, 120 population hybrids were evaluated together with their 16 parental OPVs and four checks on station at four sites: Bambey and Darou in Senegal by ISRA, and Sadoré and Tara in Niger by ISC and INRAN, respectively. Entries were randomized in an α design with three replications, 35 incomplete blocks per replicate, and four plots per incomplete block at each location. We included two location specific and two experimental cultivars, Mil de Siaka and B9 Tabi. In 2016, it was possible to test 136 population hybrids and their 17 parental OPVs (including 3/4 Ex-Borno and its offspring) at five locations: Bambey and Darou in Senegal (ISRA), and Sadoré and Tara in Niger by ISC and INRAN, respectively. Entries were randomized in an α design with three replications, 39 incomplete blocks per replicate, and four plots per incomplete block. In both years, each plot consisted of two rows (seven hills per row) of 4.8-m length, with 0.8 m between hills and 0.75 m between rows (7.2 m² plot⁻¹). Planting dates ranged from 26 July in Bambey to 25 August in Tara in 2015, and from 7 July in Sadoré to 26 July in Bambey in 2016 (Table 2). Generally, planting was too late in the rainy season of 2015 in Niger, due to operational difficulties. The Tara 2015 trial was planted next to the Niger River, due to the absence of alternatives, and additionally ~1 mo too late. This led to flooding in the early development stage and later to terminal drought stress. In contrast, the Bambey 2016 trial had sufficient precipitation, and the planting date was suitable for this region. While being rather late, end-of-July plantings are still within the range that farmers plant pearl millet in Niger and Senegal. Around the third leaf stage, plants per hill were thinned out, leaving two plants per hill. Microdosing fertilization (6 g 15–15–15 N–P–K per hill,
corresponding to \( \sim 117 \text{ kg ha}^{-1} \) was applied at planting, followed by a urea topdressing (50 kg ha\(^{-1}\)) after thinning.

The following traits were evaluated in each plot: days to 50% flowering (Julian days), plant height (cm), panicle length (cm), number of hills at harvest, number of productive panicles, and panicle yield (g m\(^{-2}\)). Seedling vigor (1 to 9, 9 being the best) was not recorded in Bambey in 2015 and in Darou in both years. Grain yield (g m\(^{-2}\)) was not recorded at Tara in 2016, and stover yield (g m\(^{-2}\)) data were not recorded at Tara and Sadoré in 2016. Thousand-grain weight was recorded only at Sadoré in 2015 and Maradi 2016. Grain Fe (kg kg\(^{-1}\)) and Zn (kg kg\(^{-1}\)) contents were analyzed only using grain from Sadoré 2016. From these data, other traits were computed, such as number of productive tillers per hill. Downy mildew pressure was almost absent at all locations in both years. It was recorded but not used in the final analysis.

Panicle yield was defined as the target trait, since grain yield was not recorded due to operational difficulties at Tara in 2016. We wanted to maintain Tara in the analysis, since each environment gives information for describing genotype \( \times \) environment (G\( \times \)E) patterns, which is crucial in the WA context. Panicle yield is a reliable proxy for grain yield \((r = 0.96, p < 0.001)\), which was confirmed by Sattler et al. (2017) and by unpublished data acquired from Pucher et al. (2016, 2015) \((r = 0.99, 0.98, \) and 0.96, respectively; \(p < 0.001)\). In addition, panicle and grain yield are influenced to the same degree by various yield components (Pucher et al., 2015, 2016; Sattler et al., 2017; present study, data not shown). Finally, WA subsistence farmers and WA breeders (Kountche et al., 2013) often rely on panicle yield as the decisive trait for cultivar selection.

### DNA Extraction and Genotyping

Nineteen seedlings from each parental population were grown in small pots at ISC in Sadoré, Niger, and irrigated manually. Leaf samples were collected from each seedling and dried using silica gel. The genomic DNA was extracted and analyzed by TraitGenetics, Germany. A set of 21 expressed sequence tag simple sequence repeat (EST-SSR) markers (three per chromosome; developed by Rajaram et al., 2013) was used (Supplemental Table S1).

### Statistical Analysis of Phenotypic Data

First, variance components were analyzed for each environment separately using the following linear mixed model:

\[
Y_{ilm} = \mu + G_i + R_l + B_{lm} + e_{ilm}
\]

where \(Y_{ilm}\) is the observed phenotype, \(\mu\) is the grand mean, \(G_i\) is the effect of genotype \(i\), \(R_l\) is the effect of replication \(l\), \(B_{lm}\) is the effect of incomplete block \(m\) nested in replication \(l\), and \(e_{ilm}\) is the residual plot variance. The adjusted mean for each entry was calculated by regarding genotypic effects as fixed, whereas the other factors were treated as random. For calculating single-environment variance components, the same model was used, but the genotype effect was treated as random. Repeatability estimates were calculated as

\[
h^2 = \frac{\sigma^2_G}{\left(\frac{\sigma^2_G + \sigma^2_I}{R}\right)}
\]

where \(\sigma^2_G\) is the genotypic variance, \(\sigma^2_I\) is the error variance, and \(R\) is the number of replications per environment. Adjusted means over all environments were calculated with the following linear mixed model assuming heterogeneous error variance of environments:

\[
Y_{iklm} = \mu + G_i + E_k + GE_{ik} + R_{il} + B_{kml} + e_{iklm}
\]

where \(Y_{iklm}\) is the observed phenotype, \(\mu\) is the grand mean, \(G_i\) is the effect of genotype \(i\), \(E_k\) is the effect of environment \(k\), \(GE_{ik}\) is the interaction effect of genotype \(i\) with environment \(k\), \(R_{il}\) is the effect of replication \(l\) in environment \(k\), \(B_{kml}\) is the effect of incomplete block \(m\) nested in replication \(l\) in environment \(k\), and \(e_{iklm}\) is the residual error variance. Genotype effect was treated as fixed, whereas all other effects were treated as random. In this study, parents are OPVs, not lines; therefore, the panmictic mid-parent heterosis (PMPH), coined by Lamkey and Edwards (1999), was calculated analogously to commonly used mid-parent heterosis. Furthermore, panmictic better-parent heterosis (PBPH) and panmictic commercial heterosis (PCH), analogous to better-parent and commercial heterosis, were used. The highest yielding parental OPV in our study, SoSat C88, was used as a benchmark to calculate PCH.

The following linear mixed model to analyze GCA and specific combining ability (SCA) variance components was designed under the assumption of heterogeneous error variance of environments:

\[
Y_{ijk}^{GCA} = \mu + \alpha + E_k + P_j + GCA_{ij} + GCA_{j} + SCA_{ij} + PE_{ij} + GCAE_{ij} + GCAE_{j} + SCAE_{ij} + R_{ij} + B_{km} + e_{ijk}
\]

where \(\alpha\) represents the average of all parents.

### Table 2. Summary of trial conditions across nine environments over 2 yr in West Africa.

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Location</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Planting date</th>
<th>Rainfall</th>
<th>Soil properties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Type</td>
</tr>
<tr>
<td>2015</td>
<td>Senegal</td>
<td>Bambey</td>
<td>14°43′12″ N</td>
<td>16°26′41″ W</td>
<td>26 July</td>
<td>662</td>
<td>Sand</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Darou</td>
<td>13°45′00″ N</td>
<td>15°48′00″ W</td>
<td>29 July</td>
<td>887</td>
<td>Sand</td>
</tr>
<tr>
<td></td>
<td>Niger</td>
<td>Sadoré</td>
<td>13°14′00″ N</td>
<td>2°17′00″ E</td>
<td>27 July</td>
<td>415</td>
<td>Seed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tara</td>
<td>11°55′24″ N</td>
<td>3°20′03″ E</td>
<td>25 August</td>
<td>364</td>
<td>Sand</td>
</tr>
<tr>
<td>2016</td>
<td>Senegal</td>
<td>Bambey</td>
<td>14°43′12″ N</td>
<td>16°36′41″ W</td>
<td>26 July</td>
<td>359</td>
<td>Sand</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Darou</td>
<td>13°45′00″ N</td>
<td>15°48′00″ W</td>
<td>22 July</td>
<td>801</td>
<td>Sand</td>
</tr>
<tr>
<td></td>
<td>Niger</td>
<td>Maradi</td>
<td>13°29′30″ N</td>
<td>7°5′47″ E</td>
<td>17 July</td>
<td>549</td>
<td>Sand</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sadoré</td>
<td>13°14′00″ N</td>
<td>2°17′00″ E</td>
<td>7 July</td>
<td>196</td>
<td>Sand</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tara</td>
<td>11°55′24″ N</td>
<td>3°20′03″ E</td>
<td>14 July</td>
<td>355</td>
<td>Sand</td>
</tr>
</tbody>
</table>

† PYD, average environmental mean for panicle yield.

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where \( Y_{i,j} \) is the observed phenotype; \( \mu \) is the grand mean, \( \alpha \) is the group effect (hybrids, parents, or checks); \( E_{i} \) is the effect of environment \( k \); \( P \) is the effect of parent \( i \); \( GCA_{i} \) and \( GCA_{j} \) are the GCA effects of parents \( i \) and \( j \), respectively, where \( i = j \); \( SCA_{ik} \) is the SCA effect of parent combination \( ij \); \( PE_{ik} \) is the interaction effect from parent \( i \) with environment \( k \); \( GCAE_{ik} \) and \( GCAE_{jk} \) are the GCA interaction effects from parents \( i \) and \( j \), respectively, where \( i = j \); \( SCAE_{ik} \) is the SCA interaction effect of hybrid \( ij \) with environment \( k \); \( B_{mlk} \) is the effect of replication \( l \) in environment \( k \); and \( e_{i,j,k} \) is the residual error variance. Group effects were treated as fixed, whereas the other factors were treated as random. Variance components significance was tested according to Stram and Lee (1994) using the likelihood ratio test for model comparison. Broad-sense heritability for population hybrids was calculated as

\[
H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GE}^2 / E + \sigma_r^2 / ER}
\]

where the total genotypic variance \( \sigma_G^2 \) was calculated as \( \sigma_{GCA}^2 + \sigma_{SCA}^2 \); genotype \( \times \) environment interaction variance \( \sigma_{GE}^2 \) was calculated as \( \sigma_{GCAE}^2 + \sigma_{SCAE}^2 \); \( E \) is the number of environments; \( \sigma_r^2 \) is the pooled error variance; and \( R \) is the number of replications per environment.

Euclidean distance was calculated based on standardized mean performances of all traits except panicle and grain yield, to avoid autocorrelations in the further analysis, and Fe and Zn, since they were only recorded in one environment. Additive main effects and multiplicative interaction (AMMI) model was computed and plotted using agricolae package (Mendiburu and Simon, 2015). All further calculations were performed using the ASReml v. 3.0 package for the R environment (Butler et al., 2009). All results were computed within the R environment version 3.2.3 (R Development Core Team, 2012).

**Statistical Analysis of Marker Data**

The number of alleles per locus, observed heterozygosity genetic distances, and association of molecular variance based on marker analysis was performed in GenAlEx V6.5 (Peakall and Smouse, 2006). Polymorphism information content (PIC) for each locus and modified Roger’s distance (MRD; Wright, 1978) were calculated based on these results. A principal coordinate analysis (PCoA) (Gower, 1966) based on MRD was used to detect population structure among the parental populations. The PCoA was performed using the APE package (Paradis et al., 2004).

**RESULTS**

**Single Trial Evaluation in Niger and Senegal**

Adjusted means per environment for panicle yield differed greatly from 21 to 477 g m\(^{-2}\) with an overall mean of 202 g m\(^{-2}\). The stress environment in Tara in 2015 led to a completely different yield level than the conditions in Bambey 2016. Here, the trial yielded on average almost six times as much as in Tara 2015 (338 vs. 63 g m\(^{-2}\)). Despite the late planting, Tara 2015 was used in the combined analysis, representing extreme stress environments. Additionally, removing Tara 2015 did not cause substantial differences in the results. All sites delivered useful data with reasonable repeatability estimates for numerous traits (Supplemental Tables S2 and S3).

**Heritability and Combining Ability Variance**

Broad-sense heritability estimates were within expectancy range. Most heritable traits were panicle length and plant height (\(H^2 = 0.94\) and 0.82). Days to 50% flowering heritability was a little lower than expected (\(H^2 = 0.68\)). The heritability estimate for the target trait panicle yield, which is largely influenced by environmental effects, was still adequate (\(H^2 = 0.53\)). The GCA variance was significant for all traits but seedling vigor. The GCA \(\times\) environment variance was significant for all traits but seedling vigor, plant height, and thousand-grain weight. The SCA variance was significant for most traits as well, except seedling vigor, and grain Fe and Zn contents. The SCA \(\times\) environment interaction variances were only significant for days to 50% flowering and panicle length. The GCA/SCA variance ratio ranged from 0.8 for thousand-grain weight to 11.8 for panicle length (Table 3). The significant positive correlation to heritability estimates (\(r = 0.60, p < 0.01\)) showed the importance of additive genetic effects for the expression of highly heritable traits.

**Superiority of Population Hybrids**

In the combined analysis, panicle yield of the population hybrids ranged from 157 to 246 g m\(^{-2}\) with an overall mean of 202 g m\(^{-2}\), whereas the per se performance of the parents ranged from 142 to 193 g m\(^{-2}\) with a mean of 164 g m\(^{-2}\). Hybrids derived from intercountry crosses yielded, on average, 202 g m\(^{-2}\), and those derived from intracountry crosses yielded 200 g m\(^{-2}\). These means were not significantly different. The best entry (panicle yield = 246 g m\(^{-2}\)) was derived from Nigerian H80-10Gr and Senegalese Thialack 2. SoSat C88 showed the highest per se performance and GCA (193 vs. 12 g m\(^{-2}\)). 3/4 Ex-Bornu had the lowest performance and GCA (142 vs. −7 g m\(^{-2}\)). The SCA effects ranged from −35 g m\(^{-2}\) for 3/4 Ex-Bornu to 11.8 for panicle length (Table 3). The average estimates of PMPH and PBPH were positive or at least equal to zero for all evaluated traits, except days to 50% flowering (−1 and −1%), panicle length (2 and −6%), grain Fe (2 and −2%), and Zn (2 and −2%) content. However, a shorter period from sowing to flowering can be desirable. Population hybrids had, on average, more tillers (12 and 8%), produced more stover (8 and 5%), had heavier grains (4 and 1%), and, particularly, had higher grain yield (23 and 18%). The PMPH for panicle yield ranged from 1 to 47% with a mean value of 23%, and the PBPH ranged from 1 to 47% with a mean of 18%. The comparison with the highest yielding parental population, SoSat C88, which served additionally as a control cultivar, revealed a PCH of 5%, on average, with a range of −19 to 28% (Table 3). The PCH regarding panicle yield relative
to the highest yielding long-panicle type, ICMV IS 89035, ranged from −15 to 34% with an average of 10%. Correlations regarding panicle yield between hybrid performance and better-parent value, PBPH%, average GCA, and SCA were significant and positive (Table 4).

We made the following assumptions based on reasonable seed costs and grain retail prices to calculate the required yield superiority of hybrids to recover the additional seed costs. A planting density of \(5 \text{ kg ha}^{-1}\) is common in Niger, and the farmers pay CFA1500 (\(\approx US\$2.58\)) for OPV and CFA2500 (\(\approx US\$4.30\)) for 1 kg of hybrid seed. Retail prices of grains are highly variable, depending on the season. We assumed an average retail price of CFA200 (\(\approx US\$0.34\)) per kilogram. This requires an additional yield superiority of 25 kg ha\(^{-1}\).

**Environmental Influence and Yield Stability**

Correlations between yield levels per environment depict large G\(\times\)E interaction effects, as already shown in the ANOVA. In 2015, correlation coefficients ranged from 0.49 between Sadoré and Tara to 0.66 between Bambey and Darou, all being significant (\(p < 0.001\)). However,
in 2016 they ranged from −0.02 between Bambey and Sadoré to 0.4 between Bambey and Darou, only half of them being significant. Interyear correlation coefficients ranged from 0.2 between Bambey 2015 and Bambey 2016 and between Sadoré 2015 and Tara 2016 to 0.38 between Sadoré 2015 and Maradi 2016 (Supplemental Table S4).

The AMMI analysis was performed without 3/4 Ex-Bornu, since this cultivar was only evaluated in 1 yr. In the analysis, Principal Component (PC) 1 explained 28.8% of the total G×E interaction. Both years of each Nigerien location, which have data from both years, Sadoré and Tara, were grouped similarly by PC1. In contrast, both years of each Senegalese location, Bambey and Darou, were separated clearly from each other. Plotting PC1 against panicle yield shows a strong negative environmental main effect in Tara in 2015, and a strong positive environmental main effect and a large G×E effect in Bambey in 2016. Maradi 2016 displayed little influence on all genotypes and showed a mean panicle yield similar to the overall panicle yield mean. Additionally, the biplot illustrates the superiority of hybrids over parental OPVs (Fig. 2). Principal Component 2 explained additional 20.6% of the total variation. Plotting PC1 vs. PC2 separated the environments even further precluding the definition of mega-environments (data not shown). Ranking accessions by AMMI stability values demonstrated the superiority of population hybrids over their parents in terms of yield stability. The highest yielding hybrid (H80-10Gr × Thialack 2) was also most stable. ICMV IS 92222 was the most stable parental OPV, ranked 63 out of 136 entries (Supplemental Table S5).

**Grouping of Parental Populations and Relation to Heterosis**

We ignored linkage disequilibrium between markers due to the rapid linkage disequilibrium decay in pearl millet (Li et al., 2010) and an even distribution of markers across the genome. Marker IPES0200 was monomorphic in most individuals and therefore removed. The applied set of EST-SSR markers detected 181 alleles, ranging from two alleles per locus for IPES0117 and IPES0174 to 20 for IPES0042 with a mean of 9.1. The PIC ranged from 0.1 for IPES0174 to 0.8 for IPES0154 with a mean value of 0.6. The observed heterozygosity was 0.5, on average, ranging from 0.1 for IPES0174 to 0.7 for IPES0206 (Supplemental Table S6). The largest amount of molecular variance, 79%, was due to the variance within populations, whereas the other sources explained only minor fractions. Variance among countries of origin was 12%, and 9% among populations. The correlations between MRD² and hybrid performance, PMPH, PBPH, GCA effects, and SCA effects were almost zero and nonsignificant (Table 4). The PCoA based on MRD calculated from SSRs could explain 27.8% of the molecular variance with the first PC and additional 13.7% with PC2. The PCoA biplot separates cultivars consistently by geographic origin. The first group contains the Senegalese cultivars, and the cultivar from the neighboring country Mauritania. The second group includes

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**Table 4. Pearson coefficients of correlation between hybrid panicle yield (PYD), panmictic mid-parent and panmictic better-parent heterosis (PMPH% and PBPH%, respectively), and average general combining ability (avg. GCA) of their parents, as well as the pairwise genetic (MRD²) and Euclidean distance (ED) between them, evaluated in 136 pearl millet hybrids and their parental populations.**

<table>
<thead>
<tr>
<th>Trait</th>
<th>PYD (hybrid)</th>
<th>PMPH%</th>
<th>PBPH%</th>
<th>Avg. GCA</th>
<th>SCA</th>
<th>MRD²</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMPH%</td>
<td>0.03ns†</td>
<td>0.19ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBPH%</td>
<td>0.57***</td>
<td></td>
<td>0.07ns</td>
<td>−0.03ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg. GCA</td>
<td>0.59***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCA</td>
<td>0.81***</td>
<td>0.08ns</td>
<td>0.72***</td>
<td></td>
<td>0ns</td>
<td></td>
</tr>
<tr>
<td>MRD²</td>
<td>−0.03ns</td>
<td>−0.12ns</td>
<td>−0.07ns</td>
<td>−0.11ns</td>
<td>0.05ns</td>
<td></td>
</tr>
<tr>
<td>ED</td>
<td>0.15ns</td>
<td>−0.07ns</td>
<td>−0.14ns</td>
<td>0.26*</td>
<td>0ns</td>
<td>0.62***</td>
</tr>
</tbody>
</table>

† ns, not significant.

*, *** Significant at the 0.05 and 0.001 probability levels, respectively.

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**Fig. 2. Additive main effects and multiplicative interaction (AMMI) biplot illustrating relationships of panicle yield level (PYD) among test sites and entries for 120 West African pearl millet population hybrids (blue), and the 16 parental populations (green) evaluated at nine environments over 2 yr (2015 and 2016). PC, principal component.**
all Nigerien cultivars. SoSat C88 and 3/4 Ex-Bornu are grouped together in the biplot (Fig. 3). Adding the third PC clearly separates those two (data not shown). However, PC3 does not cause further decisive changes.

Additionally, we calculated Euclidean distances to group parents based on phenotypic data. Finally, no significantly positive relationship between phenotypic or genotypic distance and yield level or heterosis was observed (Table 4).

**DISCUSSION**

**Variability of Environmental conditions in West Africa**

The AMMI PC1 vs. panicle yield biplot (Fig. 2) depicted distinct interactions of the trial environments with the tested genotypes. The trial in Tara 2015 was not purposely planted in a flood-prone field and 1 mo too late, but it was maintained in the data analysis to display an extreme but realistic stress environment. The two Senegalese locations in particular highlight the large genotype × year effect that is common in WA (Haussmann et al., 2012; Bashir et al., 2014a; Gemenet et al., 2014). The difference between the 2 yr shows drastically one of the major issues plant breeders have to face in WA—high interannual rainfall variability. It is arduous to develop cultivars for a large number of distinct environmental conditions. We showed that, at least on a biannual level, environments differ significantly and there is no definable or repeatable mega-environment. The importance of the large G×E interaction variance, described in this and other studies (Cooper et al., 1999; Setimela et al., 2005; Windhausen et al., 2012; Pucher et al., 2016), underlines the necessity of multilocation and multiyear testing in highly variable environments like WA to identify stable cultivars.

**Superiority of Pearl Millet Population Hybrids in Unpredictable West African Environments**

Several studies support a general superiority of pearl millet population hybrids derived from WA OPVs over established cultivars in the Sudano-Sahelian target zone. Pucher et al. (2016) reported a PMPH and PBPH for grain yield up to 73 and 69% with averages of 17 and 4%, respectively, when studying 20 WA pearl millet OPVs and their 100 offspring derived from a 10 × 10 factorial at six locations in WA in 1 yr. Ouendeba et al. (1993) used a diallel mating scheme to evaluate combining ability of five improved Sahelian landraces at two locations over 2 yr in Niger. For grain yield, they found a PBPH up to 81% with an average of 44%. Presterl and Weltzien (2003) crossed six elite cultivars based on both African and Indian cultivars, and three Indian and Pakistani landraces in a diallel mating scheme, and observed a maximum grain yield mid-parent heterosis of 30%. Our approach of studying WA population hybrids in more detail by intercrossing 17 (16 + 1) WA OPVs yielded an average PMPH of 23% with a positive minimum of 1% and an impressive maximum of 47%. The PBPH still had a range of 1 to 47%, averaging to 18%.

![Principal Coordinate Analysis based on MRD, calculated from SSRs](image)

Fig. 3. Principal coordinate analysis, estimated from modified Roger’s distance (MRD), based on 20 microsatellite markers of 17 pearl millet parental populations, originating from Mali, (brown), Mauritania (green), Niger (red), Nigeria (orange), and Senegal (blue). SSR, simple sequence repeat; PC, principal component.
However, showcasing new cultivars is only commercially meaningful in comparison with a commercially available, high-yielding cultivar. Higher seed costs have to be justified by a significant higher yield level. Therefore, PCH was calculated using SoSat C88, the highest yielding OPV in our study, as a reference. Average PCH was positive, yet very small (5%). However, our best hybrids, Nigerien H80-10Gr × Senegalese Thialack 2 and Nigerien CIVT × Malian SoSat C88 were yielding 28 and 26% more than SoSat C88. This yield superiority could easily cover the higher seed costs for hybrid seed. Average PCH of days to 50% flowering, plant height, tillering, and stover yield was small or even negative, whereas some hybrids were still outperforming the parental OPVs. The highest stover yield with a PCH of 14% was observed in Nigerien Zatib × Senegalese Thialack 2. A few hybrids had both a higher yield than SoSat C88 and a positive PCH for grain Zn and Fe content. For example Senegalese SL117 × Nigerian Ankoutess had a 9% higher panicle yield, 10% more Fe, and 38% more Zn than SoSat C88. However, grain Fe and Zn contents of more than 70 and 60 mg kg\(^{-1}\), respectively, exist in Indian pearl millet. Rather than exploring the small heterosis effects (Velu et al., 2007, 2011), introgression of such material is necessary to increase micronutrients in WA pearl millets (Pucher et al., 2014).

As mentioned in detail in the introduction, yield stability is a requirement for the success and adoption rate of new cultivars in WA. For now, population hybrids are most likely the best choice considering the highly variable environmental conditions, and the situation of smallholder farmers and seed markets in many WA countries. Heterogeneous OPVs and population hybrids, in contrast with genetically uniform single-cross hybrids, support these mechanisms and are more resistant to environmental stresses. The higher average stability of the population hybrids compared with the OPVs confirms their suitability for WA and supports the prior assumption. The highest yielding hybrid was also the most stable. This observation and the significant positive correlation between high yield and good stability in general justify striving for high-yielding population hybrids. Furthermore, progenies of population hybrids, created by random mating, will have lesser yield reduction than the offspring of single-cross hybrids. This is crucial, since farmers would rather save harvested seeds for the next rainy season than buy new material. However, higher heterosis in hybrids than in OPVs could preponderate increased seed costs, if yield stability is maintained.

**Hybrid Prediction and Heterotic Groups**

Hybrid prediction accuracy is determined by the \(\sigma_{GCA}^2 / \sigma_{SCA}^2\) ratio. A larger ratio facilitates early testing, since it enables selection of parental lines based on their offspring’s GCA (Melchinger et al., 1987). In contrast, a small \(\sigma_{GCA}^2 / \sigma_{SCA}^2\) ratio requires late testing of specific combinations to cover dominance effects. The ratio observed in our study was 0.4 for panicle yield. Thus, early testing will not lead certainly to a desired outcome. A large \(\sigma_{GCA}^2 / \sigma_{SCA}^2\) ratio is more common in single-cross (Melchinger and Gumber, 1998; Technow et al., 2014; Schrag et al., 2018) and population hybrids (Jumbo and Carena, 2008) belonging to distinct heterotic groups. A large genetic distance between the parents is supposed to increase the \(\sigma_{GCA}^2 / \sigma_{SCA}^2\) ratio, since additive variance becomes more important relative to nonadditive variance (Melchinger and Gumber, 1998; Melchinger, 1999; Schrag et al., 2006; Reif et al., 2007). Nevertheless, we observed no differences when comparing \(\sigma_{GCA}^2 / \sigma_{SCA}^2\) ratios between inter- and intracountry crosses. Pucher et al. (2016) evaluated population crosses from Burkina Faso, Mali, Niger, Nigeria, and Senegal and observed a larger \(\sigma_{GCA}^2 / \sigma_{SCA}^2\) ratio than our study. Ouendeba et al. (1993) tested population hybrids derived from landraces originating from Niger, Nigeria, Sudan, Togo, and Uganda and observed an even larger ratio. It is not possible to compare these studies directly, but the increase in \(\sigma_{GCA}^2 / \sigma_{SCA}^2\) ratio could be influenced by larger geographic and therefore genetic distances between the parents.

A direct relation between MRD\(^2\) and PMPH implies an increasing PMPH with increasing genetic distance (Falconer and Mackay, 1996; Lamkey and Edwards, 1999; Reif et al., 2005). However, there was no significant relationship between genetic distance (MRD\(^2\)) and heterosis in this study. Likewise, several other studies contradicted or could not confirm a general relationship between genetic distance and heterosis for example in maize ((*Zea mays* L.) or sorghum (*Sorghum bicolor* (L.) Moench) (Godshalk et al., 1990; Jordan et al., 2003). Chowdari et al. (1998) concluded that genetic distance based on random amplified polymorphic DNA (RAPD) and GATA binding protein 4 ([GATA]_4) microsatellite markers is not suitable for predicting heterosis in Indian pearl millet. Pucher et al. (2016) found a small yet positive and significant correlation of geographic distance to hybrid performance (0.31, \(p < 0.01\)), but not to PMPH in WA pearl millet. Gupta et al. (2018) evaluated 379 Indian pearl millet R and B lines and 51 hybrids derived from a subset of the above. They found no relationship between SSR-based simple matching distance and heterosis. The phenotype-based Euclidean distance was not standardized, thus being highly influenced by a few traits. Nevertheless, it was positively correlated to mid-parent (Set 1 = 0.59, \(p < 0.05\); Set 2 = 0.50, \(p < 0.01\)) and better-parent heterosis (Sets 1 and 2 = 0.38, \(p < 0.05\)). The relation between heterosis and genetic distance seems to be observable primarily among well-established and genetically clearly distinct groups. Apparently pearl millet cultivars are too closely related, which is a challenge for systematic and sustainable hybrid breeding.
breeding. Another reason for having no relation between genetic distance and heterosis is the set of markers used in our study. A set of neutral markers helps to distinguish different cultivars. However, only markers linked to yield-related QTLs support heterotic grouping and help to predict hybrid performance based on molecular information (Charcosset et al., 1991; Zhang et al., 1994, 1995; Jordan et al., 2003; Bashir et al., 2014b).

Defining initial heterotic groups for WA pearl millet requires a starting point. We focused on Niger and Senegal regarding selected cultivars and test environments. This was based on the two concepts of using genetically divergent groups as initial heterotic pools (Melchinger, 1999) and using geographic distances as a proxy measure for genetic distance (Moll et al., 1962), as well as first conclusions derived by Pucher et al. (2016). Their population hybrids originating from Niger × Senegal and Nigeria × Senegal crosses showed the highest yield level among all tested accessions. Melchinger and Gumber (1998) described the importance of genetic distance for heterosis performance and heterotic grouping. Larger heterosis with increasing genetic distance was observed in several crops (e.g., in maize, rye [Secale cereale L.], and oilseed rape [Brassica napus L.]). Large genetic distance was often caused by natural or anthropogenic geographic distance (Hepting, 1978; Hallauer et al., 1988; Schnell, 1992; Diers and Osborn, 1994). However, there is evidence that the level of heterosis plateaus when an optimum distance is reached and will become smaller if the distance increases further (Moll et al., 1965). Several phenotypic and genetic diversity studies concluded that strong admixture among Sahelian pearl millet cultivars is still predominant, and defining distinct groups is neither obvious nor trivial (Lewis, 2010; Stich et al., 2010; Bashir et al., 2014a; Pucher et al., 2015; Sattler et al., 2017). Despite little differences among OPVs, we were able to assign WA OPVs clearly to their country of origin based on SSRs. Regional differences (12%) and differences among cultivars (9%) explained only a fraction of the total molecular variance in our study, whereas the variance within individuals (79%) explained the largest part. This indicates high levels of allelic exchange and vast gene flow and can be explained by high outcrossing rates (Sandmeier, 1993; vom Brocke et al., 2003). Observing the largest percentage of molecular variance within, not among, groups seems to be common in Sudano-Sahelian pearl millets (vom Brocke et al., 2003; Lewis, 2010; Stich et al., 2010; Bashir et al., 2014b).

All these studies display the challenge to find heterotic groups based on naturally existing patterns in pearl millet. This is completely different from maize, where lines from distinct heterotic groups are used in hybrid breeding since the 1950s (Hallauer et al., 1988). A large differentiation between North American dent and European flint cultivars was already present in Europe at the beginning of European hybrid maize breeding (Reif et al., 2005), due to anthropogenic separation ~500 yr ago (Rebourg et al., 2003). In the United States, heterotic groups were nonexistent at the beginning of hybrid breeding (Hallauer et al., 1988) but were subsequently diverged stepwise with systematic interpopulation improvement (Duvick et al., 2004).

**Prospects and Recommendations for Future Pearl Millet Hybrid Breeding in West Africa**

Our idea of a long-term goal for pearl millet breeding is the development of hybrids from distinct heterotic groups. For now, aiming to develop topcross hybrids seems to be most promising. Single-cross hybrids are too vulnerable and the seed production is too expensive for WA. Furthermore, line development is arduous, because of the large genetic load and inbreeding depression in WA pearl millet. On the other hand, population hybrids exploit a relatively small part of the total heterosis. Topcross hybrids are also stable, exploit more heterosis than population hybrids, and have already proven their value in harsh drylands (Bidinger et al., 1994; Yadav et al., 2000; Gemenet et al., 2014). Nevertheless, a large-scale, multi-environment stability study comparing populations and topcross and single-cross hybrids in a genetically balanced setup is necessary to find the best option for WA.

Establishing heterotic groups in WA pearl millet remains challenging. Systematic target-oriented development of groups is essential, since natural patterns seem to be absent. Developing a Senegalese group around Thialack 2 and Souna 3 and a complementary Nigerien group around CIVT, H80–10Gr, and Taram seems to be promising, according to our results and those from Pucher et al. (2016). In general, their offspring are tall and have long panicles and a positive GCA regarding panicle yield. Thialack 2 and Souna 3 are both developed from Senegalese landraces. Souna 3 has proven its superior combining ability in this study and before by Pucher et al. (2016). CIVT is developed from crossing sets of S1 inbreds based on Guerguéra, and H80–10Gr is developed from crossing sets of S1 inbreds based on Guerguéra, Haini Kiri, P. Kollo, and Tamangagi (Gamoji). Taram is an improved landrace from Tara region. ICMV IS 89305 may seem to be another promising candidate. However, it was developed from Nigerien 3/4 HK B–78 and CIVT, and Senegalese Souna 3. Having a genetic background from both countries makes it unsuitable for developing a distinct group. Malian SoSat C88 does have the highest GCA regarding panicle yield but has, like its offspring, short panicles. Farmers in our target region, Niger and Senegal, prefer long panicles, which would impede farmers’ large-scale adoption of SoSat C88–derived hybrids. Intergroup reciprocal recurrent selection programs, following the example of US maize heterotic group development (Duvick et al., 2004), could help to diverge Nigerien and Senegalese
material further. Interpool improvement with emphasis on complementary alleles to the opposite pool will increase the $\sigma_{GCA}^2/\sigma_{SCA}^2$ ratio and enable early hybrid testing. While progressing, non-neutral markers might possibly support divergence of groups. Line development has to go along this process. It is important to keep the outcrossing nature of pearl millet in mind and to gradually reduce the large genetic load that is still present in WA pearl millet (e.g., via $S_1$ recurrent selection). Furthermore, it is crucial to introgress a cytoplasmic male sterility system into the pools to make the hybrid development operational simpler and, therefore, commercially feasible.

Diverging WA pearl millet groups to build the basis for sustainable hybrid breeding is laborious and will take a long time. However, population hybrids could be developed quickly and released at an early stage of the process. Thus, sharing the advantages of exploiting heterosis, at least partly, with WA farmers long before heterotic groups or topcross hybrids are established.

Focusing solely on the aspects directly related to breeding will have no impact on WA small-scale farmers. Adoption rates for OPVs are already low (5–37%) in WA (Christinck et al., 2014). Convincing farmers to buy seed of topcross hybrids regularly is even more challenging. Close collaboration between national and international institutes, seed companies, and farmers, organized in cooperatives, is crucial. A great example for successfully convincing farmers to grow hybrids in WA is sorghum (e.g., via S1 recurrent selection). Furthermore, it is crucial to introgress a cytoplasmic male sterility system into the crop science, vol. 22, july–august 2019, www.crops.org

Supplemental Material
Supplemental material is available online for this article.

Acknowledgments
The authors gratefully acknowledge the financial support of the Federal Ministry for Economic Cooperation and Development, Germany (ICRISAT-GIZ Project No. 13.1432.7-001.00, Contract No. 81170266), and the discretionary research funds provided to B.I.G. Haussmann by the McKnight Foundation Collaborative Crop Research Program. Also, the authors would like to thank the following individuals for technical assistance during seed multiplication, production of crosses, and trial implementation: A. Abarchi, H. Adamou, D. Lankoande, T. Boye, and A. Issa from ISC-Niger.

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