



# Combining ability patterns among West African pearl millet landraces and prospects for pearl millet hybrid breeding



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## ABSTRACT

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important hybrid crop in India. However, to date limited pearl millet hybrid development has been undertaken in West Africa (WA), which is the center of pearl millet origin and diversity and where this crop is most important outside India. Using a diverse set of WA pearl millet germplasm, objectives of this study were to determine the superiority of population hybrids over open-pollinated varieties for agro-morphological and agronomic traits in WA pearl millet germplasm; and (ii) to derive strategies for pearl millet hybrid breeding in WA, based on quantitative-genetic parameters, combining ability and heterotic patterns among geographically close versus distant pearl millet populations. A  $10 \times 10$  factorial mating design was performed with four parental OPVs from each of five WA countries. The 100 population hybrids and their parents were tested for 14 traits at six locations in one year, thereby using contrasting locations to indirectly sample the rainfall variability inherent to WA pearl millet production environments. Grain yield showed an average panmictic midparent heterosis (PMpH) of 16.7%, ranging from –26 to 73%. The mean grain yield of hybrids based on inter-country crosses did not differ significantly from intra-country crosses. Geographic distance between parents was positively correlated with hybrid grain yield ( $r=0.31$ ), but not with PMpH. Some crosses between accessions from Niger/Nigeria and Senegal were outstanding. Predictability of population hybrid performance for grain yield was moderate based on midparent values ( $r=0.43$ ) and slightly better based on general combining ability (GCA) ( $r=0.56$ ). Overall, pearl millet hybrid breeding in WA seems very promising, but there do not seem to be clear “natural” heterotic groups among WA pearl millet landraces. Such heterotic groups as the basis of sustainable hybrid breeding need rather to be created systematically, by building on existing combining ability patterns and aiming to maximize combining ability between the groups.

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**Abbreviations:** AMMI, Additive Main Effects and Multiplicative Interaction Model; CMS, cytoplasmic male sterility; CP, compactness of panicles; DM, percentage of downy mildew infested hills; DSY, dry stover yield; FT, days to 50% flowering;  $G \times E$ , genotype by environment; GCA, general combining ability; GY, grain yield; HI, harvest index; HP, hybrid performance; MP, midparent value; NKP, number of kernels per panicle; NPT, number of productive tillers per hill; OPV, open-pollinated variety; PC, panicle circumference; PBpH, panmictic better parent heterosis; pH, plant height; PL, panicle length;  $P_{max}$ , better performing parent; PMpH, panmictic midparent heterosis; SCA, specific combining ability; SV, seedling vigor; TKM, thousand kernel mass; VW, volumetric mass; WA, West Africa.

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## 1. Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.), the most heat tolerant and one of the most drought and salinity tolerant cultivated cereals, is a major food crop for smallholder subsistence farmers living on marginal agricultural lands in the semi-arid tropics of Africa and Asia. It is a highly cross-pollinated diploid, with  $2n = 2x = 14$  (Burton, 1974; Jauhar and Hanna, 1998), thus pearl mil-

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let landraces represent heterozygous, genetically heterogeneous open-pollinating populations.

In India, pearl millet breeding programs have been developing hybrid cultivars since the 1960s; and these hybrids have been widely adopted by Indian farmers, especially in higher yielding environments. Together with improved crop management and agricultural input use, the introduction of hybrids contributed to a grain productivity increase from 305 kg ha<sup>-1</sup> during 1951–1955 to 998 kg ha<sup>-1</sup> during 2008–2012, registering an improvement of about 200% for pearl millet in India (Dave 1986; Yadav and Rai 2013). In contrast, West African (WA) pearl millet production is still predominantly based on landraces and did not increase substantially over the last decades. Estimated adoption rates for improved open-pollinated varieties (OPVs) range from 5% to 37% depending on the study and region (Christinck et al., 2014). Although limited pearl millet hybrid breeding has been undertaken by WA breeding programs for some time, there is no viable hybrid cultivar on the seed market yet. The initial success of pearl millet hybrids in India was due to commercially-viable single cross hybrids that yielded on average 20–30% more than adapted open-pollinated varieties of similar maturity (Andrews and Kumar 1992). Grain yields of hybrids have subsequently increased at >2% per year whereas those of OPVs stagnated (Yadav and Rai 2013) resulting in widespread adoption of hybrids in agro-ecologies where private-sector and/or public-sector breeding has focused on hybrids. Previous studies on African material reported about population hybrid performance. To describe the difference between a population hybrid and the mean of its two parental populations, the term panmictic midparent heterosis (PMpH) was introduced by Lamkey and Edwards (1999). This term will be used in the following to reflect the difference to the “conventional” midparent heterosis based on single crosses derived from homozygous inbred lines. Ouendeba et al. (1993) reported for African pearl millet a mean yield PMpH of 55% for a set of 10 WA population hybrids, while a mean heterosis of 8% was found by Bidinger et al. (2005) for Eastern African topcross hybrids.

Identification of heterotic patterns is the fundamental step for effective hybrid breeding seeking to exploit heterosis. In theory, heterotic groups are genetically distant to each other, are defined by their high combining ability when crossed, and should not be intercrossed for hybrid parent improvement (Melchinger and Gumber 1998). In some Indian pearl millet hybrid breeding programs, genotypes were not always explicitly allocated to either the female or male heterotic group, thus genetic intermixture has decreased the genetic distance between groups (Gupta et al., 2015). To avoid such inconsistencies in hybrid development, it is important to identify heterotic groups, where genotypes designated to one group will never be crossed to the other group for line development.

In WA, there have been only very limited systematic studies on population hybrids including diverse pearl millet populations or OPVs as parents. Previous pearl millet studies using genotypic and phenotypic data generally showed that genetically clearly distinct groups do not exist for WA pearl millet germplasm, rather a high degree of admixture was found (Bashir et al., 2014; Hu et al., 2015; Pucher et al., 2015). Similarly, Stich et al. (2010) did not observe clearly distinct clusters although they identified five subgroups using the software STRUCTURE (Pritchard et al., 2000) within a collection of 145 WA inbred lines genotyped with SSR markers. Gemenet et al. (2015) observed three subgroups in a diverse set of 155 WA pearl millet inbred lines based on DArT markers. The generally high degree of admixture found in the WA pearl millet germplasm is explained by the high outcrossing rate, variable planting dates, robust pollen, and long duration of the flowering growth stage within landraces and improved OPVs (Hausmann et al., 2007; Lakis et al., 2012), which results in overlapping flowering periods and gene flow even between early and late germplasm. Thus identification of initial putative heterotic

groups does not seem straightforward by means of genetic diversity analysis. Although pearl millet is highly outcrossing, natural pollen flow should be localized within a certain geographic radius, thus a relation between genetic and geographic distance of landraces might be suspected. However, only a low association between geographic distance and dissimilarity measures has been detected. For example significant but low correlations were found by Pucher et al. (2015) using phenotypic data ( $r=0.18$ ), and by Mariac et al. (2006) using 25 microsatellite markers ( $r=0.11$ ). Similarly, based on whole-genome surveys with GBS markers, Hu et al. (2015) observed low but significant degrees of genetic similarity (as indicated by low  $F_{ST}$  values for accessions from two different countries) among pearl millet accessions originally collected from parts of Southern and Eastern Africa, and from Southwestern and South Asia, whereas higher  $F_{ST}$  values were observed between accessions from Senegal and those from surveyed countries in Southern and Eastern Africa and South Asia. The lowest observed  $F_{ST}$  values were observed when comparing accessions from Zimbabwe and South Africa. However, verification of the relationship between geographic distance of parental materials and heterotic response of the corresponding crosses is missing for pearl millet. Nonetheless, such relationships have supported heterotic grouping in other crops e.g. maize and ryegrass (Zheng et al., 2008; Posselt 2010).

Efficient breeding programs require an optimum allocation of resources, which is strongly dependent on the ratio of the genetic variance components, their environmental interactions, and the residual variance (Gordillo and Geiger 2008; Tomerius et al., 2008). Thus these quantitative-genetic parameters should be investigated at the beginning of a hybrid breeding program. Also the relationships between grain yield and other agro-morphological and phenological traits need to be investigated to enable analyses of trade-offs, a better understanding of mechanisms of adaptation and also to understand farmer preferences better.

So the main objectives of the present study were (i) to determine the superiority of population hybrids over open-pollinated varieties for agro-morphological and agronomic traits in WA pearl millet germplasm; and (ii) to derive strategies for pearl millet hybrid breeding in WA, based on quantitative-genetic parameters, combining ability and heterotic patterns among geographically close versus distant pearl millet populations.

## 2. Materials and methods

### 2.1. Plant materials

Four pearl millet populations (landraces or improved OPVs) each from five WA countries (Senegal, Mali, Burkina Faso, Niger and Nigeria) with early to medium maturity were randomly selected to develop 100 population hybrids using a ten × ten factorial mating design. The populations were part of a collection that was created jointly by the Institut de la Recherche pour le Développement (IRD) and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) during collection missions in 1976 and 2003. One earlier and one later flowering population/OPV from each country were used as female parents, and similarly for the male parents. Flowering time information was based on passport data from the accessions, and did not always hold true in our test and seed production environments. The 100 population crosses therefore represented intra-country and inter-country population crosses with variable geographic distance and partially variable flowering times among the parental populations. The population crosses were created in the irrigated off-season nursery of 2005/2006 at the ICRISAT Sahelian Center research station at Sadoré (Niger) using three sowing dates and profiting from the strong protogyny of pearl millet. At least 25 panicles from each female parent were pollinated

with a pollen bulk of the respective male parent to avoid genotypic sampling effects in the parental populations and to produce enough seed of each population hybrid for use in multi-location trials.

Population hybrids, as well as the 20 parental populations and one widely-adapted control variety (SOSAT-C88) were tested on-station at six locations in WA in 2006. The locations were Bambey, Senegal (Senegalese Institute for Agricultural Research, ISRA); Samanko, Mali (ICRISAT); Cinzana, Mali (Institute of Rural Economy, IER); Gampela, Burkina Faso (Institute of the Environment and Agricultural Research, INERA); Sadoré, Niger (ICRISAT); and Maiduguri, Nigeria (Lake Chad Research Institute, LCRI). The sites differed in soil type, date of sowing, total rainfall and its distribution (Table 1). The reason of using these contrasting sites was to indirectly sample the tremendous inter-annual rainfall variability inherent to WA pearl millet production environments. While the Samanko site is geographically outside the actual target area for the tested germplasm, similar amounts of rainfall can be received in some years in the actual target environment of the Sahelian-Soudanean zone of WA.

At each location the trial was laid out in an  $\alpha$ -design with three replications, eleven incomplete blocks per replicate, and eleven plots per incomplete block. Each plot had two rows of 4.8 m length and 0.75 m inter-row distance, and alleys were 1.2 m wide. Within rows, hills with two plants each were grown at 0.8 m intervals, so each plot consisted of 14 hills occupying 9 m<sup>2</sup> and grown at a density of 15,556 hills ha<sup>-1</sup>.

Phenotypic traits evaluated on a 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 hills, number of productive tillers, grain yield (g m<sup>-2</sup>), dry stover yield (g m<sup>-2</sup>), thousand kernel mass (g), compactness of panicle (rating from 1 to 3, 1 = not compact, 3 = very compact) and volumetric mass of grains (g L<sup>-1</sup>). The latter trait is of both farmers' and consumers' interest, as retail market prices for pearl millet grain are often based on volumetric units. Plant height (cm), panicle length (cm), and panicle circumference (cm) were measured on main-stem culms of three representative plants per plot, and the average taken forward for analysis.

Due to operational difficulties, volumetric mass of grains was not recorded at Samanko and Maiduguri, and dry stover yield was not recorded at Samanko.

The number of kernels per panicle was estimated based on the formula: (grain yield  $\times$  thousand kernel mass)/(number of productive tillers per plot  $\times$  1000). Harvest index was estimated as the percentage of grain yield to total above-ground biomass yield (= dry panicle yield + dry stover yield).

## 2.2. Statistical analysis

The observed data was analyzed by a one-step approach, including hybrid and parental population performance from all six environments. The following model was used to estimate the adjusted means:

$$y_{ikmn} = \mu + g_i + l_k + gl_{ik} + r_{mk} + b_{nk} + e_{ijkmn}$$

where  $y_{ikmn}$  is the phenotypic mean of the  $i$ th entry in the  $n$ th incomplete block in the  $m$ th replication at the  $k$ th location,  $\mu$  denotes the overall mean,  $g_i$  the genotypic effect of the  $i$ th entry,  $l_k$  the location effect of the  $k$ th location,  $gl_{ik}$  the genotype by environment interaction effect of the  $i$ th entry and the  $k$ th location,  $r_{mk}$  the effect of the  $m$ th replication at the  $k$ th location,  $b_{nk}$  the effect of the  $n$ th block in the  $k$ th location and  $e_{ijkmn}$  was the residual. Except the genotypic effect, all effects were treated as random. To determine whether inter-country crosses were yielding significantly different

from intra-country crosses, we added one parameter in the model, which distinguished between those two levels.

Estimation of variance components was done using the following model:

$$y_{ijkmn} = \mu + l_k + a + p_i + p_j + g'_i + g'_j + s_{ij} + (pl)_{ik} + (pl)_{jk} + (g'l)_{ik} + (g'l)_{jk} + (sl)_{ijk} + r_{mk} + b_{nk} + e_{ijkmn}$$

where  $y_{ijkmn}$  is the phenotypic mean of the  $ij$ th entry (parent  $i = j$ , or hybrid  $i \neq j$ ) at the  $k$ th location in the  $m$ th replication in the  $n$ th incomplete block,  $\mu$  denotes the overall mean,  $l_k$  the location effect of the  $k$ th location,  $a$  the genetic group effect (hybrids, males, females, and the controls each had a separate level),  $p_i$  and  $p_j$  the effect of the  $i$ th female and  $j$ th male parental populations, respectively,  $g'_i$  the general combining ability (GCA) effect of the  $i$ th female parent,  $g'_j$  the GCA effect of the  $j$ th male parent,  $s_{ij}$  the specific combining ability (SCA) effect of the cross between parents  $i$  and  $j$ ,  $(g'l)_{ik}$  and  $(g'l)_{jk}$  were GCA  $\times$  location interaction effects of the female and male parental population, respectively,  $(sl)_{ijk}$  the SCA  $\times$  location interaction effects,  $r_{mk}$  the effect of the  $m$ th replication at the  $k$ th location,  $b_{nk}$  the effect of the  $n$ th block in the  $k$ th location and  $e_{ijkmn}$  was the residual. Dummy variables were used to separate genotypes into parental populations and hybrids as described by Piepho et al. (2008). Except the group effect all effects were treated as random. Error variances were assumed to be heterogeneous among locations.

Significance of the variance components was tested by model comparison with likelihood ratio tests, where the full model was tested against the reduced model, which excludes the variance component of interest. The halved  $p$  values were used as approximation (Stram and Lee 1994). Broad-sense heritability on the entry-mean basis was calculated for female parents, male parents and hybrids separately as  $H^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_{G \times E}^2 / l + \sigma_e^2 / lr)$ , where  $\sigma_G^2$  and  $\sigma_{G \times E}^2$  refer to the total genotypic variance of the female or male parents and the corresponding G  $\times$  E interaction variances, respectively,  $l$  to the number of locations,  $r$  to the number of replications and  $\sigma_e^2$  to the residual variance. For heritabilities of hybrids, the total genotypic variance was calculated as  $\sigma_G^2 = \sigma_{GCA-F}^2 + \sigma_{GCA-M}^2 + \sigma_{SCA}^2$ , and the G  $\times$  E interaction variances as  $\sigma_{G \times E}^2 = \sigma_{GCA-F \times E}^2 + \sigma_{GCA-M \times E}^2 + \sigma_{SCA \times E}^2$ .

The ratios of the GCA variance components to the SCA variance components is an indicator for the prediction accuracy based on GCA effects (Reif et al., 2013) and were calculated as  $(\sigma_{GCA-F}^2 + \sigma_{GCA-M}^2) / \sigma_{SCA}^2$ .

To determine the GCA and SCA effects separately for each location, we modified the above shown mixed model, by excluding the location factor and all factors including an interaction with the location.

For each combination of parental populations, midparent value (MP), relative PMpH and relative panmictic better parent heterosis (PBpH) were calculated using hybrid performance (HP) as follows:  $MP = (P1 + P2) / 2$ ,  $PMpH = [(HP - MP) / MP] \times 100$ ,  $PBpH = [(HP - Pmax) / Pmax] \times 100$ , where  $Pmax$  is the better performing parent.

For flowering time and percentage of downy mildew incidence, we defined the parent with the lower value as the better parent, and made a change of sign for PMpH and PBpH.

Pearson's correlation was calculated across all environments between MP and HP,  $r(MP, HP)$ , between SCA and HP,  $r(SCA, HP)$ , between better parent performance (BP) and HP,  $r(BP, HP)$ , and between HP and the sum of the GCA effects of both parents  $r(GCA, HP)$  using the leave-one-out validation as explained by Schrag et al. (2009).

To determine the coherency of the geographic distance between the origins of the two parents and the performance of the hybrid and the PMpH effect, we correlated the geographic distance of the

**Table 1**  
Information and mean grain yield for experimental sites.

| Site           | Latitude | Longitude | Altitude (m) | Soil type      | Average annual rainfall (mm) | Rain in 2006 (May–October) |              | Date of sowing (all in 2006) | Mean grain yield ( $\text{g m}^{-2}$ ) |
|----------------|----------|-----------|--------------|----------------|------------------------------|----------------------------|--------------|------------------------------|--|
|                |          |           |              |                |                              | Total (mm)                 | N rainy days |                              |  |
| Bambey (SN)    | 14.709   | −16.479   | 20           | Sandy          | 611                          | 479                        | 39           | 26/07                        | 70                                     |
| Samanko (ML)   | 12.517   | −8.067    | 326          | Loamy ferrasol | 1018                         | 1008                       | 63           | 30/06                        | 80                                     |
| Cinzana (ML)   | 13.254   | −5.968    | 282          | Sandy loam     | 630                          | 759                        | 58           | 22/07                        | 152                                    |
| Gampela (BF)   | 12.419   | −1.351    | 275          | Sandy loam     | 815                          | 751                        | 51           | 13/07                        | 108                                    |
| Sadoré (NR)    | 13.238   | 2.280     | 235          | Sandy          | 556                          | 561                        | 40           | 15/07                        | 49                                     |
| Maiduguri (NA) | 11.835   | 13.152    | 320          | Sandy          | 621                          | 600                        | 45           | 14/07                        | 93                                     |

Source of average rainfall: <http://www.worldclimate.com/>, assessment period varies between locations (minimum of 60 years).

SN = Senegal, ML = Mali; BF = Burkina Faso; NR = Niger; NA = Nigeria.

parents' origin with the HP and PMpH values of the corresponding hybrid. The morphological data was used to compute a putative measure of genetic distance between parents. Mean performances of all traits except grain yield, and those having a higher correlation than 0.6 with grain yield (number of kernels per panicle and dry stover yield), were standardized and used to create a dissimilarity matrix based on Euclidean distance method. The distance obtained is termed morphological distance. All statistical computations were done using the R software package, stability analysis and the Additive Main Effects and Multiplicative Interaction Model (AMMI) plots were done using the package agricolae (de Mendiburu 2015), and mixed models analyses were performed using the software ASReml-R 3.0 (Butler et al., 2009).

### 3. Results

#### 3.1. Environmental means and data quality at the individual test sites

Grain yield levels of the six environments differed considerably with environmental means ranging from  $49 \text{ g m}^{-2}$  at Sadoré to  $152 \text{ g m}^{-2}$  at Cinzana, indicating high stress level differences between the experimental sites (Table 1). The Cinzana environment was particularly outstanding with  $44.2 \text{ g m}^{-2}$  greater mean grain yield than the second best environment Gampela. The higher grain yields observed at Cinzana might partly be related to the relatively high number of rainy days at this location during the 2006 growing season (Table 1).

Repeatabilities for grain yield for the six environments ranged between 0.43 and 0.74 (Supplemental Table 2). In comparison, for the highly heritable trait flowering time we observed repeatabilities between 0.79 (Bambey) and 0.95 (Gampela and Sadoré).

#### 3.2. Hybrid means, extent of heterosis, and correlations among traits

Mean grain yields of the population hybrids showed a high variation, ranging from  $48.8$  to  $122.6 \text{ g m}^{-2}$ . The parental populations *per se* performance ranged from  $49.1$  to  $116.4 \text{ g m}^{-2}$  (Table 2, for data from the individual environments, see Supplemental Table 1).

Hybrids based on intra-country crosses showed a mean grain yield of  $91.0 \text{ g m}^{-2}$ , while inter-country crosses showed with  $95.4 \text{ g m}^{-2}$  a numerically higher, though not significantly greater mean grain yield. Except for two parents (M06, F02 and F03), the marginal means of the hybrid parents were higher than their *per se* performance. Parent F08 especially showed a high hybrid advantage, yielding on average  $102.1 \text{ g m}^{-2}$  as hybrid parent, but only  $62.1 \text{ g m}^{-2}$  as a population *per se*. The male parent M01 (Souna3 from Senegal) showed with  $105.7 \text{ g m}^{-2}$  the highest marginal mean of all parents, while its *per se* performance was with  $89.1 \text{ g m}^{-2}$  only a bit higher than the average *per se* performance of all parents. The female parent F02 (PE03012, also from Senegal) showed

a very good marginal mean and the highest *per se* performance with  $116.4 \text{ g m}^{-2}$  (Table 2).

The two best hybrid combinations, F02  $\times$  M10 and F08  $\times$  M01, yielding  $122.6$  and  $122.4 \text{ g m}^{-2}$  were based on crosses between geographical distant parental populations originating from Senegal and Nigeria, and Senegal and Niger, respectively.

Hybrids and parents showed similar means and variation for panicle length, and panicle circumference, but difference in mean and/or variation for most other traits such as seedling vigor, days to flowering, number of productive tillers per hill, grain yield, and compactness of panicle (Fig. 1). Especially, hybrids showed higher maximum values compared to the parents for seedling vigor, number of productive tillers, grain yield, harvest index, thousand grain mass and grain volumetric mass, and lower minimum values for flowering time. Especially for harvest index and number of productive tillers per hill, the best hybrids were far better than the best parent genotypes (with one exception for number of productive tillers per hill). Such differences in the maximum values indicate the potential superiority of hybrids compared to population varieties through selection.

The PMPH of hybrids for grain yield was on average 16.7% and ranged from  $-25.6\%$  to  $73.2\%$  for individual hybrids (Table 3). The average PBPH was 3.9% and ranged from  $-32.1$  to  $69.0\%$ . The control variety SOSAT-C88 had a grain yield of  $98.0 \text{ g m}^{-2}$ , which results in a commercial heterosis of 25% for the two best hybrids.

The average PMPH differed across environments ranged between 4.0% at Samanko and 47.3% at Bambey, while there was no significant correlation between the PMPH and the mean grain yield of the environments.

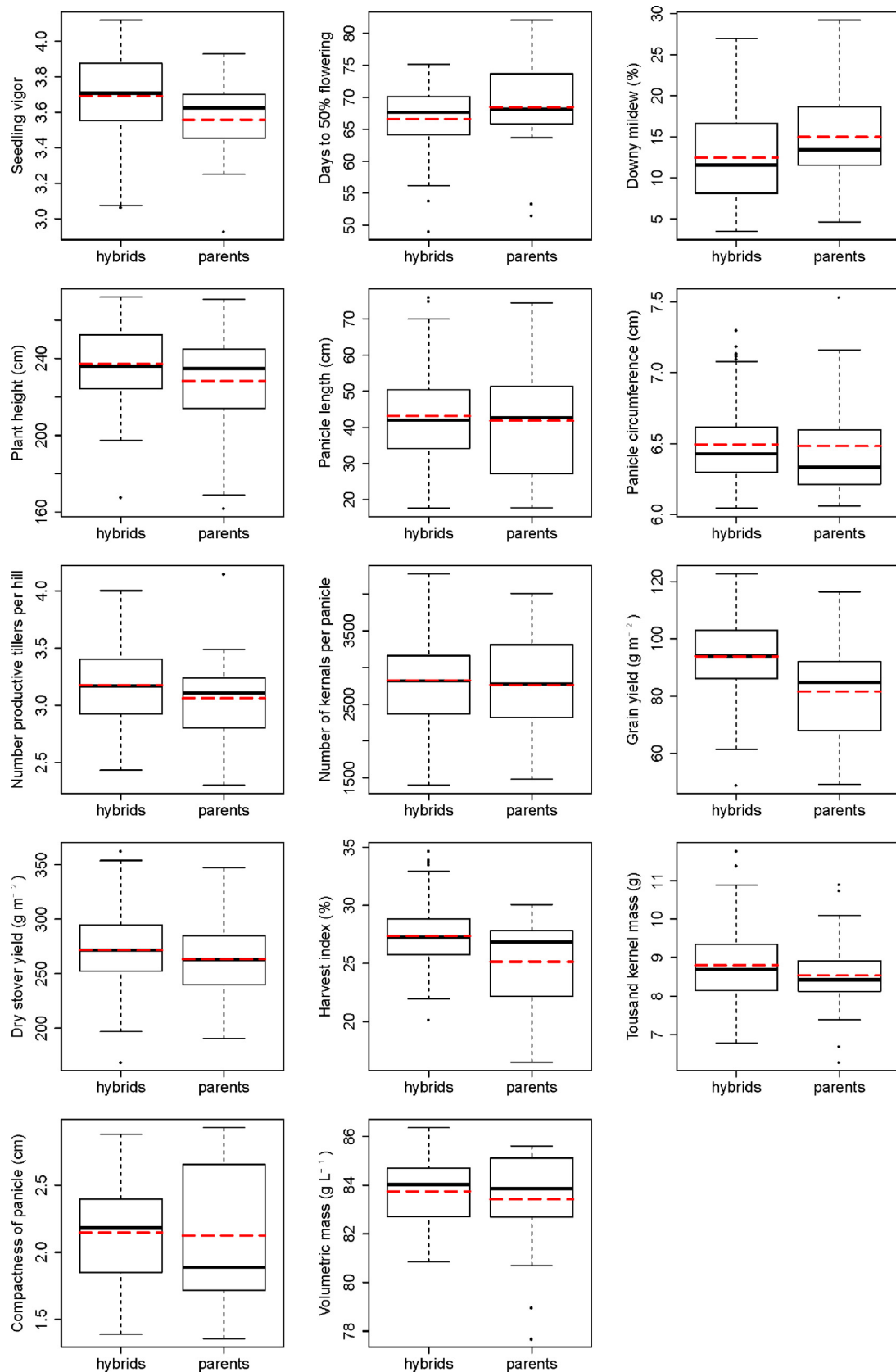
Beside grain yield, downy mildew reaction showed a high mean PMPH (16.9%), indicating an average lower incidence level of hybrids compared to parental means, while the PBPH was on average negative ( $-15.9\%$ ). Further, harvest index showed a relative high PMPH (9.6%), while PMPH estimates for other traits were ranging between  $-5.3$  and 4.0% (Table 1).

Correlations between grain yield and the yield component traits panicle length, panicle circumference and number of productive tillers were rather low ranging from  $r = -0.15$  to 0.33 (Supplemental Table 4). In comparison, number of kernels per panicle showed a high correlation of ( $r = 0.61$ ), while this might partly be explained by an autocorrelation, because the traits was derived by other traits including grain yield, and not independently determined. Thousand kernel weight was negatively correlated with grain yield ( $r = 0.30$ ) and compactness of panicles ( $r = -0.46$ ), while compactness was positively correlated with grain yield ( $r = 0.38$ ).

#### 3.3. Genetic variance components and heritabilities

Combined across the six test sites, female and male parental populations and hybrids showed significant genetic variance and a  $G \times E$  interaction effect (Table 3) for most traits.





**Fig. 1.** Box plots, including the arithmetic mean (red dashed line) for 15 traits evaluated on 100 pearl millet population hybrids and their 20 parents, combined across six locations in West Africa. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 2**  
Mean grain yield ( $\text{g m}^{-2}$ ) of 100 pearl millet population hybrids (green color coding illustrates the yield level), derived from crossing open-pollinated landraces or improved OPV cultivars from five different countries; marginal means for each hybrid parent (purple color coding); and the performance *per se* of the 20 parental populations (orange color coding), evaluated at six locations in Nigeria, Niger, Burkina Faso, Mali and Senegal in 2006.

| Female                    | Male          | Senegal      |               | Mali          |               | Burkina Faso  |               | Niger         |               | Nigeria      |              | Mean  | Female parent <i>per se</i> |
|---------------------------|---------------|--------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|--------------|--------------|-------|-----------------------------|
|                           |               | M01 (Souna3) | M02 (PE01437) | M03 (PE05393) | M04 (PE05558) | M05 (PE05988) | M06 (PE05980) | M07 (PE02827) | M08 (PE02885) | M09 (AON644) | M10 (AON514) |       |                             |
| Senegal                   | F01 (PE02990) | 97.2         | 101.4         | 95.2          | 91.7          | 79.6          | 99.8          | 102.7         | 98.1          | 87.3         | 99.1         | 95.2  | 91.6                        |
|                           | F02 (PE03012) | 88.6         | 112.2         | 94.3          | 109.3         | 84.8          | 110.7         | 97.9          | 113.0         | 104.8        | 122.6        | 103.8 | 116.4                       |
| Mali                      | F03 (PE05532) | 111.8        | 87.5          | 91.8          | 92.9          | 78.9          | 85.8          | 75.3          | 99.5          | 91.4         | 97.9         | 91.3  | 98.6                        |
|                           | F04 (PE05631) | 114.3        | 89.5          | 104.8         | 100.3         | 86.5          | 76.1          | 95.3          | 86.0          | 84.9         | 108.3        | 94.6  | 92.4                        |
| Burkina Faso              | F05 (PE05996) | 90.0         | 80.3          | 90.8          | 61.4          | 48.8          | 81.5          | 81.2          | 101.3         | 71.2         | 68.3         | 77.5  | 49.1                        |
|                           | F06 (PE01203) | 115.5        | 91.3          | 112.8         | 100.3         | 91.9          | 93.5          | 86.1          | 92.5          | 107.3        | 103.3        | 99.5  | 85.7                        |
| Niger                     | F07 (PE02801) | 90.1         | 94.6          | 103.4         | 90.0          | 76.7          | 94.3          | 84.7          | 76.3          | 81.6         | 96.5         | 88.8  | 51.5                        |
|                           | F08 (PE02935) | 122.4        | 96.9          | 108.6         | 110.3         | 78.6          | 102.5         | 95.2          | 101.7         | 93.6         | 110.7        | 102.1 | 62.1                        |
| Nigeria                   | F09 (AON378)  | 117.4        | 88.4          | 89.3          | 97.5          | 71.9          | 89.4          | 112.6         | 90.1          | 90.0         | 71.8         | 91.8  | 85.8                        |
|                           | F10 (AON122)  | 109.9        | 103.9         | 104.0         | 71.5          | 76.4          | 97.3          | 95.5          | 93.8          | 73.6         | 112.4        | 93.8  | 83.8                        |
| Mean                      |               | 105.7        | 94.6          | 99.5          | 92.5          | 77.4          | 93.1          | 92.6          | 95.2          | 88.6         | 99.1         | 93.8  | 81.7                        |
| Male parent <i>per se</i> |               | 89.1         | 92.4          | 82.5          | 65.3          | 52.5          | 112.1         | 70.6          | 90.2          | 78.7         | 81.6         | 81.5  |                             |

Heritabilities of female and male parental populations and hybrids showed a very wide range across the different traits. Panicle length, with  $h^2 = 0.99$  and  $0.98$  for parents and hybrids, respectively, was the most heritable trait. Grain yield showed reasonable heritability values of  $0.82$  and  $0.65$  for the female and male parental populations, respectively, and a value of  $0.61$  for the hybrids. Downy mildew incidence, which is generally less heritable due to high  $G \times E$  interaction, showed heritabilities between  $0.33$  and  $0.36$  for hybrids and parents.

Differences among GCA effects of the female and male parents were significant for most traits except for seedling vigor and downy mildew incidence. In case of grain yield, the  $GCA_{\text{Male}}$  effects, and for harvest index the  $GCA_{\text{Female}}$  effects were not significant. For most traits  $GCA \times E$  interactions were significant, except  $GCA_{\text{Female} \times E}$  for panicle length, thousand kernel mass, and volumetric mass, and  $GCA_{\text{Male} \times E}$  for seedling vigor. The SCA effects were significant for most traits as well, except for downy mildew incidence, number of productive tillers per hill and volumetric mass. Genetic variance components estimated for each location separately showed, that there were differences among locations, *i.e.*, the tested materials did not reveal significant differences for all traits in all test sites (Supplemental Table 5). However, for grain yield, genetic differences among the parents and their  $GCA_{\text{Male}}$  and  $GCA_{\text{Female}}$  effects were significant at most locations.

The  $\sigma^2_{GCA}/\sigma^2_{SCA}$  ratio for the different traits ranged from  $1.0$  for volumetric mass to  $32.5$  for panicle length and correlated significantly with the trait heritabilities ( $r = 0.63$ ), which indicates that highly heritable traits are determined predominantly by additive effects.

From the observed ratios, it can be concluded that the traits flowering time, plant height, panicle length, and number of kernels per panicle were largely determined by additive genetic effects, while dominance effects were relatively important for the traits number of productive tillers, grain yield, harvest index, and volumetric grain mass.

#### 3.4. Relationship among hybrid performance, heterosis, GCA, SCA and geographic distance

The correlations between HP and the mean GCA of both parents (based on the leave one out method) were highly significant and above  $0.63$  for all traits except seedling vigor ( $r = 0.2$ ,  $p < 0.05$ , Table 3). The highest correlation was found for panicle length ( $r = 0.97$ ). For grain yield we found for HP vs. GCA a correlation of  $r = 0.56$ . The correlations between HP and MP were significant

for all traits and similar but generally a bit lower than correlations between HP and GCA. Correlations between BP and HP were for most traits smaller than  $r(\text{GCA}, \text{HP})$ , while for downy mildew and grain yield, the BP showed a higher correlation to HP than GCA. Correlations between HP and SCA were also significant for all traits, but in most cases at a lower level than  $r(\text{GCA}, \text{HP})$ . The highest correlations between HP and SCA were found for grain yield and number of productive tillers, *i.e.*, for traits with a low  $\sigma^2_{GCA}/\sigma^2_{SCA}$  ratio.

The significant correlation ( $r = 0.74$ ) between  $\sigma^2_{GCA}/\sigma^2_{SCA}$  and  $r(\text{GCA}, \text{HP})$ , and the negative correlation ( $r = -0.75$ ) between  $\sigma^2_{GCA}/\sigma^2_{SCA}$  and  $r(\text{SCA}, \text{HP})$  indicate that the higher the influence of the additive effects, the better will be the accuracy of HP prediction using GCA. In case of grain yield, the higher correlation between SCA and HP, compared to GCA and HP, and the low  $\sigma^2_{GCA}/\sigma^2_{SCA}$  ratio confirm the relatively high influence of dominance effects for this trait.

Geographic distances between parents' origins were significantly positively correlated with hybrid grain yields ( $r = 0.31$ ), number of kernels per panicle ( $r = 0.24$ ) and volumetric mass ( $r = 0.32$ ). Geographic distances between parents and PMpH estimates were not significantly correlated for any trait. Morphological distance, as a putative measure for genetic distance between parents, showed no significant correlation with HP or PMpH (Supplemental Table 4).

#### 3.5. Patterns of $G \times E$ interaction

The extent of  $G \times E$  interaction compared to the genetic variance was relatively large, especially for the traits downy mildew incidence, grain yield, number of productive tillers, and harvest index. Coefficients of correlation among the test sites for grain yield ranged from  $0.16$  (non-significant) to  $0.52$  (significant at  $p < 0.01$ ; Supplemental Table S6). The location Maiduguri revealed in general the lowest correlations with the other test environments for grain yield. Significant, moderate to high positive correlations among all test sites were obtained for the following traits: days to 50% flowering, plant height, panicle length, panicle circumference, thousand kernel mass, volumetric mass, and number of kernels per panicle. Correlations among sites were highly variable and partially negative for downy mildew incidence (Fig. 1).

In the AMMI analysis for grain yield, PC1 and PC2 explained  $35.4\%$  and  $26.3\%$ , respectively, of the total variation (Fig. 2). The plot showed that the vectors for environments Sadoré, Gampela, and Maiduguri point in similar directions, indicating relatively low  $G \times E$  interaction between these environments. The other three

**Table 3**

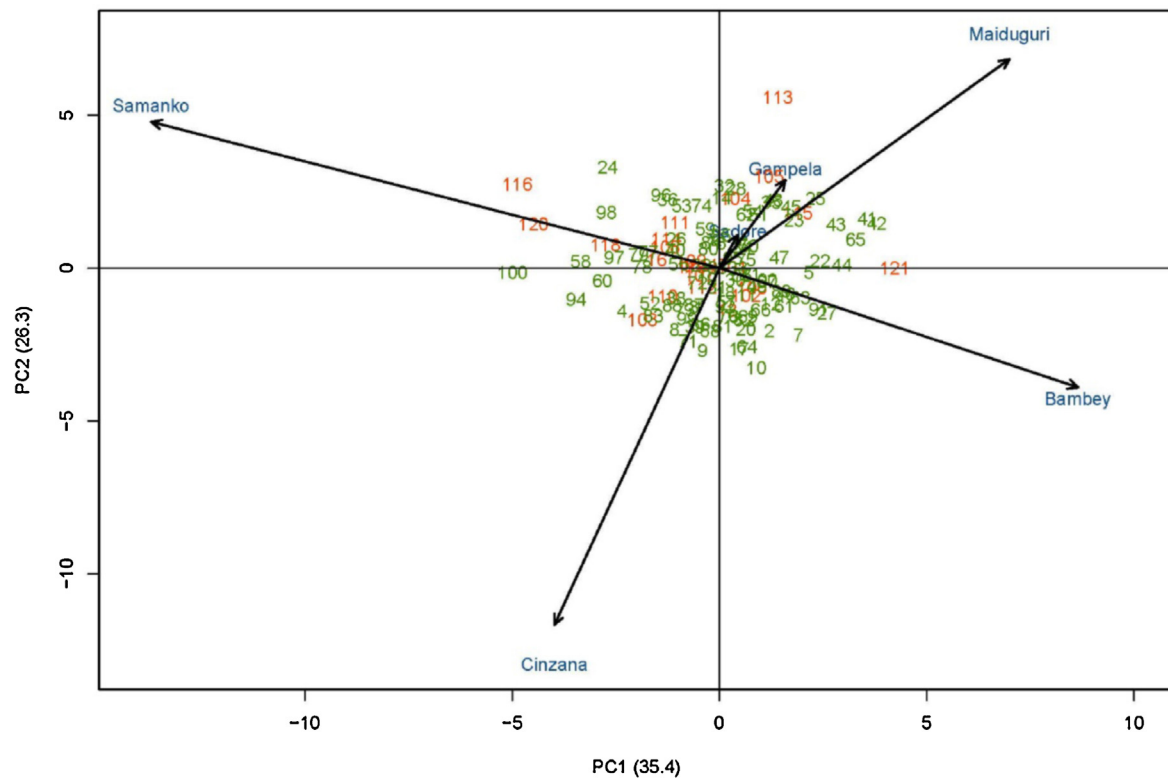
Estimated variance components, heritabilities, mean and range of panmictic midparent (PMpH) and panmictic better-parent heterosis (PBpH), theratio of GCA vs. SCA as well as correlations between general combining ability effects and hybrid performance  $r(\text{GCA}, \text{HP})$  (for traits where  $\sigma^2_{\text{GCA}}$  is significant), midparent value and hybrid performance  $r(\text{MP}, \text{HP})$ , better parent value and hybrid performance  $r(\text{MBP}, \text{HP})$ , specific combining ability effects and hybrid performance  $r(\text{SCA}, \text{HP})$  (for traits where  $\sigma^2_{\text{SCA}}$  is significant), geographic distance between origin of parental populations and hybrid performance  $r(\text{geo.dist}, \text{HP})$ , geographic distance and panmictic midparent heterosis (PMpH), and effects estimated across six locations in WA.

| Parents                                    | SV               | FT             | DM (%)           | pH (cm)        | PL (cm)         | PC (cm)        | NPT             | NKP             | GY (g m <sup>-1</sup> ) | DSY (g m <sup>-1</sup> ) | HI (%)          | TKM (g)         | CP              | VM (g L <sup>-1</sup> ) |
|--|------------------|----------------|------------------|----------------|-----------------|----------------|-----------------|-----------------|-------------------------|--------------------------|-----------------|-----------------|-----------------|-------------------------|
| $\sigma^2_{\text{G-Female}}$               | 0                | 31.34**        | 17.02            | 666.2**        | 234.74**        | 0.16**         | 0.18**          | 572213**        | 404.4**                 | 585.1                    | 10.17**         | 1.54**          | 0.31**          | 1.81**                  |
| $\sigma^2_{\text{G-Male}}$                 | 0.03             | 64.19**        | 15.96            | 902.0**        | 278.08**        | 0.13**         | 0.01            | 473713**        | 198.8*                  | 1361.9*                  | 10.88*          | 1.13**          | 0.22**          | 7.63**                  |
| $\sigma^2_{\text{Female} \times \text{E}}$ | 0.07*            | 2.86**         | 145.98**         | 31.8           | 0               | 0.05           | 0.18**          | 146870**        | 255.5**                 | 560.8                    | 6.48*           | 0.14            | 0.07*           | 1.28                    |
| $\sigma^2_{\text{Male} \times \text{E}}$   | 0.06*            | 6.12**         | 150.02**         | 280.5**        | 4.89*           | 0.03           | 0.09*           | 41718           | 359.5**                 | 842.6                    | 17.47**         | 0.37**          | 0.01            | 0.41*                   |
| $h^2_{\text{Female}}$                      | 0                | 0.95           | 0.35             | 0.96           | 0.99            | 0.79           | 0.68            | 0.84            | 0.82                    | 0.44                     | 0.73            | 0.94            | 0.91            | 0.75                    |
| $h^2_{\text{Male}}$                        | 0.45             | 0.97           | 0.33             | 0.93           | 0.99            | 0.77           | 0.16            | 0.83            | 0.65                    | 0.63                     | 0.65            | 0.89            | 0.92            | 0.95                    |
| Hybrids                                    |                  |                |                  |                |                 |                |                 |                 |                         |                          |                 |                 |                 |                         |
| $\sigma^2_{\text{GCA-Female}}$             | 0.01             | 8.78**         | 3.87             | 109.1**        | 56.80**         | 0.06**         | 0.02*           | 168057**        | 45.9**                  | 370.6**                  | 0               | 0.48**          | 0.08**          | 0.38**                  |
| $\sigma^2_{\text{GCA-Male}}$               | 0                | 17.75**        | 4.86             | 263.3**        | 88.10**         | 0.03**         | 0.03**          | 159459**        | 33.60                   | 453.8**                  | 3.44**          | 0.34**          | 0.03**          | 1.58**                  |
| $\sigma^2_{\text{SCA}}$                    | 0                | 1.71**         | 1.73             | 12.5*          | 4.46**          | 0.01**         | 0.02**          | 15394**         | 45.65**                 | 148.8**                  | 0.87            | 0.08**          | 0.01*           | 2.02                    |
| $\sigma^2_{\text{GCA-F} \times \text{E}}$  | 0.02**           | 0.56**         | 40.38**          | 21.1**         | 0.18            | 0.05**         | 0.03**          | 29997**         | 48.0**                  | 313.9**                  | 2.40**          | 0.01            | 0.01**          | 0                       |
| $\sigma^2_{\text{GCA-M} \times \text{E}}$  | 0                | 3.69**         | 19.54**          | 104.6**        | 1.21**          | 0.01**         | 0.04**          | 77908**         | 138.0**                 | 223.8**                  | 3.64**          | 0.04*           | 0.02**          | 0.17**                  |
| $\sigma^2_{\text{SCA} \times \text{E}}$    | 0                | 0.24           | 6.29**           | 17.9           | 2.21**          | 0              | 0               | 3750            | 5.5                     | 0                        | 1.42            | 0.06            | 0.01            | 0.05                    |
| $h^2$                                      | 0.44             | 0.94           | 0.36             | 0.89           | 0.98            | 0.69           | 0.51            | 0.76            | 0.61                    | 0.57                     | 0.52            | 0.9             | 0.84            | 0.95                    |
| PMpH (%) mean                              | 3.8              | 2.6            | 16.9             | 4.0            | 3.1             | 0.2            | 4.0             | 2.4             | 16.7                    | 3.2                      | 9.6             | 3.2             | -0.4            | 0.4                     |
| Range                                      | (-15.1 to 17.9)  | (-4.8 to 12.4) | (-115.8 to 57.4) | (-4.4 to 13.4) | (-13.3 to 36.1) | (-7.3 to 8.4)  | (-17.9 to 25)   | (-23 to 38.9)   | (-25.6 to 73.2)         | (-16.5 to 30.2)          | (-16.8 to 45.7) | (-9.1 to 18.7)  | (-37.2 to 30.7) | (-1.4 to 2.5)           |
| PBpH (%) mean                              | -0.5             | 8.3            | -15.9            | -2.7           | -13.5           | -2.8           | -2.5            | -10.4           | 3.9                     | -4.4                     | 0.3             | -3.6            | -13.5           | -1.1                    |
| Range                                      | (-15.3 to 15.29) | (-0.2 to 26.9) | (-293.1 to 44.7) | (-15.1 to 8.4) | (-42.4 to 24.5) | (-12.2 to 6.0) | (-30.9 to 23.5) | (-47.3 to 23.7) | (-32.1 to 69.0)         | (-34.5 to 29.0)          | (-28.7 to 32.9) | (-19.1 to 15.1) | (-46.8 to 25.2) | (-4 to 1.5)             |
| GCA/SCA                                    | -                | 15.5           | 5.0              | 29.8           | 32.5            | 9.0            | 2.5             | 21.3            | 1.7                     | 5.5                      | 4.0             | 10.3            | 11.0            | 1.0                     |
| $r(\text{GCA}, \text{HP})$                 | -                | 0.94***        | -                | 0.94***        | 0.97***         | 0.78***        | 0.64***         | 0.90***         | 0.56***                 | 0.71***                  | 0.68***         | 0.88***         | 0.85***         | 0.89***                 |
| $r(\text{MP}, \text{HP})$                  | 0.26**           | 0.92***        | 0.75***          | 0.90***        | 0.96***         | 0.78***        | 0.55***         | 0.84***         | 0.43***                 | 0.69***                  | 0.44***         | 0.86***         | 0.81***         | 0.88***                 |
| $r(\text{BP}, \text{HP})$                  | 0.21*            | 0.61***        | 0.8***           | 0.75***        | 0.8***          | 0.77***        | 0.40**          | 0.66***         | 0.32***                 | 0.52***                  | 0.32***         | 0.76***         | 0.69***         | 0.62**                  |
| $r(\text{SCA}, \text{HP})$                 | -                | 0.34***        | -                | 0.34***        | 0.24***         | 0.59***        | 0.73***         | 0.41***         | 0.77***                 | 0.66***                  | -               | 0.47***         | 0.51***         | -                       |
| $r(\text{geo.dist}, \text{HP})$            | 0.01             | -0.02          | -0.23*           | 0              | 0.03            | -0.08          | 0.16            | 0.24*           | 0.31**                  | 0.12                     | 0.19            | -0.26**         | -0.32**         | 0.32*                   |
| $r(\text{geo.dist}, \text{PMpH})$          | 0.01             | -0.15          | -0.18            | -0.05          | 0.1             | 0.08           | 0.03            | 0.1             | 0.07                    | 0.09                     | 0.05            | 0.08            | 0               | -0.08                   |

Traits are: SV Seedling vigor, FT Days to 50% flowering, DM Percentage of downy mildew infested hills, pH Plant height, PL Panicle length, PC Panicle circumference, NPT Number of productive tillers per hill, NKP Number of kernels per panicle, GY Grain yield, DSY Dry stover yield, HI Harvest index, TKM Thousand kernel mass, CP Compactness of panicles, VM Volumetric mass.

GCA/SCA was calculated as  $(\sigma^2_{\text{GCA-F}} + \sigma^2_{\text{GCA-M}}) / \sigma^2_{\text{SCA}}$ .

Significance levels are: \* significant at  $p < 0.05$ , \*\* significant at  $p < 0.01$ , \*\*\* significant at  $p < 0.001$ , respectively.



**Fig. 2.** AMMI biplot for 100 pearl millet population hybrids (green numbers) and their 20 parental populations (red numbers) tested at six environments in West Africa (blue names and vectors), showing the grain yield stability of genotypes and the relationships among test locations and genotypes. For assignment of entry numbers, see Supplemental Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

sites, Cinzana, Samanko and Bambe, each pointed to a different direction, which indicates that the ranking of the genotypes was different in each of these sites. Since data are based on one year only, it is not possible to assign sites to repeatable mega-environments in this study. During the 2006 rainy season, Samanko received the highest rainfall of the six environments and had also the highest number of rainy days (Table 1). Such differences in rainfall could be a reason for the separation of these environments in the AMMI plot. The AMMI plot for downy mildew incidence also showed a separation of Cinzana, and a grouping of Maiduguri and Gampela (pointing to the opposite direction as Cinzana), suggesting that there are important differences in the virulence of the pathogen causing this pearl millet disease across the test environments used in this study, while the pattern of the other three locations differed compared to the AMMI plot for grain yield (Supplemental Fig. 1).

The GCA effects of the 20 parents, determined for grain yield and downy mildew incidence at each location separately (Supplemental Table 7), gave hints about the stability of the GCA effects. While the combining ability effects varied between locations and were generally very small for downy mildew incidence, parents with a good GCA for grain yield combined over locations, had generally also a good combining ability at most or all individual locations.

The AMMI plot also illustrates grain yield stability of the genotypes across the six environments. Stable genotypes are located around the center point ( $PC1 = 0$  and  $PC2 = 0$ ), and unstable genotypes are more distant to this center point, e.g. entry 116 (parent M06), which is closest to the endpoint of the vector for Samanko, had a relatively high grain yield at that location compared to the other environments. The most-widely adapted entry, (hybrid F02  $\times$  M10), was placed in the center of the AMMI plot, indicating high stability over all locations.

## 4. Discussion and conclusion

### 4.1. The prospects of pearl millet hybrids in west africa

Our study presents data on population hybrids based on WA pearl millet germplasm, tested in WA. Our results can also be of broader interest for the worldwide pearl millet breeding community, especially in India, since an increased understanding of heterotic patterns in the crop's center of diversity will give the opportunity to diversify the germplasm in other pearl millet growing regions. Especially well advanced hybrid breeding programs could seek for introgression of new material.

The average of 17% PMpH and the maximum of 73.2% PMpH for grain yield observed in our study suggest a good potential for pearl millet hybrids to contribute to enhanced pearl millet productivity in the Sudano-Sahelian agro-ecologies of WA, and were within the range of heterosis effects found in previous studies on pearl millet. Ouendeba et al. (1993) found a better parent heterosis effect of up to 81% for hybrids based on African landraces in a two years study conducted at two locations in Niger. A lower maximum heterosis of 30% was observed by Presterl and Weltzien (2003) in population hybrids based on Indian and African landraces and population varieties during a study conducted in eight environments in India. Bidinger et al. (2003) studied the extent of heterosis in pearl millet topcross hybrids, which were based on seed parents (A-lines) and landrace pollinators of primarily Indian germplasm. They observed panmictic heterosis values of  $-11$  to  $+17\%$  for grain yield and of  $-26$  to  $+6\%$  for stover yield (in relation to the landrace pollinator), which was slightly smaller than observed in our study. Although not directly comparable with our results, Gemenet et al. (2014) reported for 155 WA pearl millet testcross hybrids (one inbred tester crossed with 155 partial inbred lines derived from WA landraces) under low and high soil phosphorous conditions mean



midparent heterosis values of 43.5% and 39.2%, respectively, and a maximum heterosis value of 98.1%. Those studies including ours, indicate the general superiority of pearl millet hybrids in various environments.

Maize, the prime example for a hybrid crop, has shown in some studies a similar or higher extent of PMpH than what was found in our study. For instance, [Carena \(2005\)](#) found for maize population hybrids an average PMpH of 20% across environments, and [Reif et al. \(2003\)](#) reported based on several experiments on subtropical maize population hybrids in different environments (Mexico, Columbia and Thailand) a mean PMpH of 48%. Although a real comparison of the two crops is not possible, because the extent of heterosis is highly dependent on the tested material chosen within each trial, it indicates a similar hybrid potential of pearl millet and maize.

The superiority of hybrids over the better parent is of interest to determine the effective heterosis and benefit of growing a hybrid compared to the better parental population. In our study the hybrids with the highest and the second highest grain yields showed a PBpH of 5.3% (F02 × M10) and 37.3% (F08 × M01), respectively, indicating a yield increase through hybrids, especially for the latter one. However, across the 2006 rainy season test environments used for this study, the better parental population (F02, which is also the best of all parental population) of the F02 × M10 cross would be a better current option for farmers for the time being, as its yield averaged only 5% less than the best population hybrid tested. So the cost of purchasing fresh hybrid seed each season (and risks associated with such cash outlays for resource-poor farmers) is likely to be greater than the benefits obtained, unless it proves possible for farmers to cost-effectively use the Syn<sub>1</sub> generation of the population hybrid (and thereby spread the seed purchase cost across 2 crop seasons). For the short-to-medium term it would be useful to compare the F<sub>1</sub> (=Syn<sub>0</sub>) and F<sub>2</sub> (=Syn<sub>1</sub>) generations of the best population hybrids with certified seed (R<sub>1</sub> or R<sub>2</sub> generation) and farmer-saved seed (harvested from grain production plots sown with R<sub>1</sub> or R<sub>2</sub> seed) of their parental populations.

Comparison of the best performing hybrids with a control variety is needed to determine the hybrid superiority over existing improved OPVs (also known as commercial heterosis), which indicates the economic benefit for the farmer. In our trial, the hybrid superiority over the control OPV “SOSAT-C88” was 25% for the two best hybrids. However, it has to be mentioned that only one control OPV was included in our trial, and its flowering time was marginally earlier than most of the populations hybrids evaluated in this trial, thus a sound conclusion on the commercial heterosis cannot be drawn here. However, the improved pearl millet OPV Souna 3, which is recommended for cultivation in the national catalogues of Senegal and Niger is included as a parental population. This entry can be used to assess the commercial heterosis of the best population hybrids tested at the experimental location of Senegal and Niger (Bambey and Sadoré). For the 10 best hybrids the superiority over Souna 3 was ranging from 30 to 66%, which indicates the high benefit through heterosis and potential of hybrids.

In comparison to the other traits observed, grain yield showed the highest average PMpH (for traits other than downy mildew incidence) and PBpH, which is in line with other studies on pearl millet ([Bidingier et al., 2003](#); [Yadav 2006](#)) and other crops ([Niehaus and Pickett 1966](#); [Lippman and Zamir 2007](#); [Longin et al., 2013](#)). Those studies reported generally the highest extent of heterosis for the complex trait grain yield and lower levels for yield component traits. The observed relatively small positive PMpH for plant height compared to grain yield, and heterosis towards early flowering has also been found by [Ouendeba et al. \(1993\)](#) in pearl millet, and can be explained by the relatively high heterozygosity level of the parental populations (not showing much inbreeding depression). So midparent heterosis for plant height would be expected to be much larger if inbred lines had been used as hybrid parents, par-

ticularly if the parents had complementary alleles at one or more of the major dwarfing gene loci known in this species ([Appa Rao et al., 1986](#); [Chowdari et al., 1998](#)).

Beside hybrid grain and stover yield potentials, the stability of hybrid performance under varying climate conditions is of importance, especially in WA. Population hybrids as well as topcross hybrids have a higher inter-variety heterogeneity and therefore a higher population buffering potential than genetically uniform single cross hybrids ([Hausmann et al., 2012](#)). But hybrid seed production is generally based on a cytoplasmic male sterile (CMS) parents. Since production of CMS female parents is operationally simpler using inbred lines, topcross hybrids may be more practical than population hybrids. However, [Rai et al. \(2000\)](#) reported development of an A<sub>4</sub> cytoplasm population A<sub>4</sub>/B<sub>4</sub> pair in pearl millet based on 2–3 cycles of S<sub>1</sub> recurrent selection (with testcrosses to an A<sub>4</sub> cytoplasm male-sterile line to assess sterility maintenance capacity of each S<sub>0</sub> plant in each cycle) in the genetic background of the Nigerian Dwarf Composite, NCD<sub>2</sub> ([Rai et al., 1995](#)). The B<sub>4</sub> maintainer population for this pearl millet seed parent was registered in Crop Science, so both CMS-based and non-CMS-based population hybrid seed production systems will be worthwhile exploring while taking forward the findings of the current study for possible commercialization of pearl millet hybrids in WA.

To benefit from promising population hybrids found in this study, without the requirement of a CMS system in the short term, multiplication of those hybrids by random mating could be a rewarding approach. Although progenies are expected to have a little lower grain yield compared to the initial population hybrid due to recombination, this yield loss will be less compared to yield reduction in progenies from single-cross hybrids. Hardy-Weinberg equilibrium will be reached after one generation of random mating, and the new population will remain superior to the initial parental populations. Thus the use of such population crosses as new varieties will be a great option to get a fast benefit from the observed panmictic midparent heterosis.

#### 4.2. Heterotic patterns and predictability of hybrid performance

In our study, hybrid combinations with one parent originating from Senegal and one from Niger or Nigeria showed in several cases a very high grain yield. Because of this, we found that population hybrid performance was significantly positively correlated with geographic distance of OPV origin ( $r=0.3$ ). However, PMpH was not significantly correlated with geographic distance. Therefore, a direct relationship between the heterosis effect and geographic distance was not confirmed, and putative heterotic groups cannot be based solely on the countries of origin of the parental populations. This was confirmed by other results from this study: although inter-country crosses yielded slightly more than inter-country crosses, this difference was not statistically significant. Further, the morphological distance as a measure of genetic distance showed no association with HP or PMpH, which could indicate that there is no strong association between genetic distance and heterosis. But it needs to be considered that morphological distance based on 11 traits cannot be equalized with genetic distance. Even though the approach of heterotic grouping based on the genetic distance has been suggested in several previous studies ([Melchinger and Gumber 1998](#); [Wu et al., 1999](#); [Reif et al., 2003](#)), it was identified as an unfavorable approach by others ([Edmands 2002](#); [Yu et al., 2005](#)). [Chowdari et al. \(1998\)](#) studied the association between genetic distance and hybrid performance, heterosis over mid parent, and heterosis over the better parent using Indian pearl millet inbred lines and could not find any significant correlation. This indicates that in pearl millet, heterotic grouping based on combining ability could be more efficient than a grouping based on genetic distances.

An additional study using advanced genotyping methods and diallel crosses is presently underway to verify this.

An effective identification and selection of superior hybrid combinations is crucial in a hybrid breeding program. Several studies have shown that predicting hybrid performance by the GCA effects is more accurate than prediction by midparent values (Gowda et al., 2012; Guo et al., 2013), which is in line with our study, and can be explained with the dominance effects that are completely ignored if midparent values are used (Xu and Zhu 1999).

For grain yield and number of productive tillers, the higher correlation between SCA and HP ( $r=0.77$ ), compared to GCA and HP ( $r=0.56$ ), points to the relatively lower influence of additive compared to dominance effects, since SCA is mainly determined by the latter. The relative low ratio of GCA/SCA (1.7) also confirms the high level of dominance effects for grain yield, which is unfavorable for the prediction of hybrid performance based on GCA. This means that a two-step selection procedure might be envisaged. After pre-selection of potential hybrid parents based on GCA, evaluation of factorial crosses among the best combiners in the female and male pools is required to identify the best performing hybrids based on both GCA and SCA. According to Melchinger et al. (1987), the establishment of heterotic groups increases the  $\sigma^2_{GCA}/\sigma^2_{SCA}$  ratio; this might be one way to increase the prediction accuracy of using GCA in the longer term.

#### 4.3. Importance of multi-location testing

When discussing hybrid breeding, the aspect of  $G \times E$  and yield stability needs to be considered in addition to mean yield potential. Pearl millet farmers in WA must contend with substantial high climate and rainfall variability, which requires pearl millet cultivars with high ability of population buffering capacity (Haussmann et al., 2012). The inter-annual rainfall variability was also visible in our study, where the locations Bambey, Gampela and Cinzana received within 2006 a largely different level of rainfall compared to the long-term average rainfall at these sites (Table 1).

In our study, grain yield stability of population hybrids and their parental populations (landraces and OPVs) across a wide range of environments was analyzed using an AMMI biplot, which showed that hybrids and parents had similar ranges of stability. This hints that population hybrids and parents show similar  $G \times E$  interactions and buffering capacity in diverse environments, unless specifically selected for traits conferring yield stability. However, it is clear that such population hybrids would have greater population buffering capacity than any single-cross hybrid grown in pure stand (but physical mixtures of single-cross hybrids could well be comparable to population hybrids and their parental populations in this regard).

We found strong  $G \times E$  interactions in the grain yield AMMI plot (Fig. 2), which underline the importance of selecting for yield stability. Specific adaptation to mega-environments could also be considered, but identification of repeatable mega-environments requires data from multiple years which were not available in this study. Downy mildew incidence, which is also strongly influenced by  $G \times E$  interaction (in this case due to virulence differences in pathogen populations present in the different testing environments), showed similarities, but also differences compared to the pattern of environmental grouping in the grain yield AMMI biplot (Supplemental Fig. 1). Analysis of multi-year multi-locational trials and continuous monitoring of environmental patterns over several years should be a good approach to identify reliable mega-environments, which would preferably be suitable for yield performance testing as well as for downy mildew screening. This would enable site-specific breeding and higher selection gains for the target region. Pucher et al. (2015) characterized 360 WA pearl millet landraces at six environments in 2006, where five environments overlapped with our study, and studied the correlations

between environments. They found that Cinzana had relatively low correlation to the other environments for grain yield, which would be in line with the pattern observed in our AMMI analysis.

The ratio of the genetic variance components, the  $G \times E$  interaction variance, and the residual variance is of high interest, enabling an optimum allocation of resources in a breeding program. The observed ratio of  $\sigma^2_{GCA}$  vs  $\sigma^2_{GCA \times E}$  of 0.42 for grain yield in this study indicates a high degree of  $G \times E$  interaction and therefore the importance of multi-location multi-year testing, in order to identify repeatable mega-environments and exploit specific adaptation to subsets of the test environments, or otherwise to select specifically for buffering capacity and enhanced yield stability. If GCA values of combining ability trials are used to predict untested hybrid combinations, then the level of  $G \times E$  interaction and the possibility of distinct mega-environments should be considered.

#### 4.4. Can hybrid breeding in west africa produce real benefits for smallholder farmers?

Due to the significant PBpH observed in the present and prior studies, pearl millet hybrid cultivars hold great potential as a means to help increase pearl millet productivity in WA. However, hybrid cultivars are not necessarily an uncontested favorable technology for smallholder farmers. Many people believe that hybrid varieties need more intensely managed growing conditions to perform well. However, the experience of smallholder pearl millet producers in India, where >70% of the 9Mha pearl millet area is sown with freshly purchased single-cross hybrid seed (Yadav and Rai, 2013), suggests that this is not necessarily the case. In our study, the extend of PMpH was not related to the environmental mean for grain yield, so there was no clear indication under which environmental conditions (low- versus high-yielding) population hybrids are potentially more advantageous. Yadav et al. (2000) tested top-cross hybrids in two different drought stress environments and one wet environment in India over three years, and found generally a higher heterosis for grain yield in the drought stress environments, while on average the heterosis was 30%. Similarly, Gemenet et al. (2014) reported marginally higher midparent heterosis for pearl millet testcross hybrids under low-phosphorus soil conditions (43.5%) than under high-phosphorus soil conditions (39.2%) across several years and locations in WA. Further, a study on sorghum single-cross hybrids showed hybrid superiority of up to 37% for grain yield, relative to a well-adapted control OPV, tested on 27 farmers' fields in Mali (Rattunde et al., 2013). This hybrid superiority was consistently expressed in both low-yielding and high-yielding environments. Ultimately, the performance and superiority of hybrids in low-input or stress-prone environments depends upon the genetic materials used in creation of the hybrids and how they (and/or their ancestral germplasm) were selected (i.e., under high- or low-input conditions).

Experience with sorghum hybrids in Mali has shown that farmers' seed cooperatives can be trained to produce hybrid seeds and multiply the parental materials, as well as how to effectively market the hybrid seed that they produce. This strategy has sparked a great interest in hybrid seed production among farmers across several different parts of Mali. At present, farmer seed producers in selected areas in Mali are excited to enter sorghum hybrid seed production because of the opportunity to combine a "cash-crop" (seed harvested from the female parent can be sold on the market) and a "food-crop" (grain harvested from the male parent can be used for consumption) in the same field (Weltzien, personal communication). Similar approaches could be used to deploy population cross and topcross hybrids of pearl millet, with the male parental population being harvested for grain from the seed production plots before the female parent (inbred or population, male-sterile or male-fertile) is harvested as a seed crop.

#### 4.5. Conclusions for pearl millet hybrid breeding in west africa

The estimated values of up to 73% and 69% PMHP and PBpH, respectively, for WA population hybrids, illustrate that exploitation of heterosis in future WA pearl millet breeding programs can be potentially very rewarding. However, data from more years are required to further validate this finding, to identify repeatable mega-environments and exploit specific adaptation patterns in WA hybrid breeding.

Clear grouping of germplasm to define heterotic groups remains a challenge, because of the high levels of genetic admixture of WA pearl millet germplasm (due to a combination of protogyny-facilitated outcrossing and extremely robust wind- and insect-borne pollen). Hence genetically clearly distinct groups do not yet exist for WA pearl millet, and grouping based on geographic distance or country of origin do not seem to be appropriate approaches either. Studies using genotypic data and data of combining ability studies would be needed to determine the real relation between genetic distance and the heterosis effect, to develop further strategies. Despite the fact that there was no clear combining ability pattern for certain country combinations, our study indicated that crosses between populations from Senegal and Niger or Nigeria generated some outstanding hybrids. Division of a portion of the potential parental materials from these two geographically distant regions into a pair of gene pools targeted for reciprocal recurrent selection to enhance both productivity *per se* of the gene pools as well as heterosis of crosses between the two gene pools, appears to be an option worth testing, and could be underpinned by studying the genetic distance. To delimit the various options exploiting this germplasm in further hybrid breeding, it seems promising to include material from Senegal, Niger and Nigeria in future projects on combining ability to first define heterotic groups and then bring the benefit of heterosis as soon as possible to WA pearl millet farmers. In the longer term, a systematic approach to establish heterotic groups based on combining ability pattern trials seems to be most promising. The low GCA/SCA ratio for grain yield at present decreases the predictability of hybrids by GCA values, thus a two-step selection procedure based on both GCA and SCA might be preferable, at least in the medium term. Furthermore, alternative prediction methods like genomic tools might be an option in future pearl millet hybrid breeding for WA. Regional cooperation and germplasm exchange among WA countries will need to be strengthened in the context of pearl millet hybrid breeding. This will enable the establishment of well adapted and genetically diverse heterotic parental pools that can be exploited to benefit pearl millet-producing smallholder farmers (and pearl millet consumers) across the region.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fcr.2016.04.035>.

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