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Heterosis in locally adapted sorghum genotypes and potential of hybrids for increased productivity in contrasting environments in Ethiopia

Taye T. Mindaye[a,b,*], *Emma S. Mace*[c], *Ian D. Godwin*[d], *David R. Jordan*[a]

[a]*The University of Queensland, Queensland, Alliance for Agriculture and Food Innovation, Hermitage Research Facility, 604 Yangan Rd, Warwick, QLD, 4370, Australia*
[b]*Ethiopian Institute of Agricultural Research, Melkassa Research Center, P O Box 436, Nazareth, Ethiopia*
[c]*Department of Agriculture and Fisheries, Hermitage Research Facility, 604 Yangan Rd, Warwick, QLD, 4370, Australia*
[d]*The University of Queensland, School of Agriculture and Food Sciences, Brisbane, QLD, 4072, Australia*

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ABSTRACT

Increased productivity in sorghum has been achieved in the developed world using hybrids. Despite their yield advantage, introduced hybrids have not been adopted in Ethiopia due to the lack of adaptive traits, their short plant stature and small grain size. This study was conducted to investigate hybrid performance and the magnitude of heterosis of locally adapted genotypes in addition to introduced hybrids in three contrasting environments in Ethiopia. In total, 139 hybrids, derived from introduced seed parents crossed with locally adapted genotypes and introduced R lines, were evaluated. Overall, the hybrids matured earlier than the adapted parents, but had higher grain yield, plant height, grain number and grain weight in all environments. The lowland adapted hybrids displayed a mean better parent heterosis (BPH) of 19%, equating to 1160 kg ha^{-1} and a 29% mean increase in grain yield, in addition to increased plant height and grain weight, in comparison to the hybrids derived from the introduced R lines. The mean BPH for grain yield for the highland adapted hybrids was 16% in the highland and 52% in the intermediate environment equating to 698 kg ha^{-1} and 2031 kg ha^{-1}, respectively, in addition to increased grain weight. The magnitude of heterosis observed for each hybrid group was related to the genetic distance between the parental lines. The majority of hybrids also showed superiority over the standard check varieties. In general, hybrids from locally adapted genotypes were superior in grain yield, plant height and grain weight compared to the high parents and introduced hybrids indicating the potential for hybrids to increase productivity while addressing farmers' required traits.

Abbreviations: BLUP, Best linear unbiased predictor; BPH, best parent heterosis; DTF, days to flowering; GN, grain number head^{-1}; GW, hundred grain weight; GY, grain yield; HLA, highland adapted hybrids; IRL, introduced R line hybrids; LLA, lowland adapted hybrids; PTH, plant height.

* *Corresponding author at:* Ethiopian Institute of Agricultural Research, Melkassa Research Center, P O Box 436, Nazareth, Ethiopia.
E-mail address: tayabo@gmail.com (T.T. Mindaye).

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1. Introduction

Sorghum (*Sorghum bicolor* L. Moench) is a C_4 cereal crop domesticated in Africa; it is adapted to water stress, low soil fertility and high temperature conditions. *Sorghum* is a staple crop for more than 500 million people in 30 sub-Saharan African and Asian countries [1], while it is primarily grown as feed grain in the developed world.

In Ethiopia, which is the sixth largest sorghum producing country in the world, sorghum contributes 17% of the total annual cereal grain production [1,2]. It is grown in highly diverse environments, which can be broadly classified into three major agro-ecologies; highland areas >1900 m, intermediate areas between 1600 and 1900 m and lowlands areas <1600 m above sea level, characterized by distinct edaphic and climatic conditions [3]. Sorghum productivity is constrained by different biotic and abiotic factors mainly drought and *Striga* (a parasitic weed) in the lowland and biotic stress in the highland and intermediate environments.

Sorghum is predominantly grown by smallholder farmers in Ethiopia. The highest proportion (74%) of the grain produced is consumed at the household level, with the remainder being used for sale and seed purposes [2]. The grain is used for preparation of different local staple food products such as leavened bread (*injera*), porridge and local beverages that require specific grain quality characters. Grain size and color are important traits to farmers in selecting varieties [4]. Increased grain size with corneous endosperm is preferred and larger seeded varieties fetch a better price, possibly due to higher milling yields and higher water absorbance [5]. The stover, which has uses for animal feed, fuel and construction of fences, is often valued as highly as grain yield, hence taller varieties are highly preferred by farmers [4,6]. However, the improved varieties released in Ethiopia to date have had very low adoption rates. Lack of farmers preferred traits in these released varieties is the major impediment to their wider adoption [4,7]. The majority (85%) of the improved varieties released for use in the lowland and intermediate environments were developed using lines introduced from outside of Ethiopia; these are characterized by short plant stature, early maturity and lower grain size [4,8]. All varieties released for the highland environment to date have been pure lines selected from highland landrace collections; however, these released improved varieties only have limited yield advantage compared to the farmers' selected varieties or landraces [4].

The demand for improved varieties with both higher grain yield and farmer's preferred traits, primarily grain size and plant height, is increasing due to the rapidly growing human population and changing standard of living. Hybrid technology could have the potential to increase productivity while retaining high biomass and large grain size. Sorghum hybrids have been grown by farmers in developed countries since the late 1950s after the discovery of a viable cytoplasmic male sterility system, allowing cost-effective hybrid production, and are increasingly being adopted in the developing world [1]. In sorghum superiority of the F_1, or hybrid vigour, can result in a 30–40% increase in grain yield, depending on the environment and the genotypes used [9,10]. In addition to increasing yield, sorghum hybrid vigor has also been demonstrated to have increased yield stability over inbred lines, particularly in stressed environments [10,11].

Efficient and successful hybrid breeding requires the development of complementary parental pools [12]. In a mature hybrid crop breeding system such as maize, this has involved the development of genetically divergent parental pools that combine consistently to produce high yielding hybrids. While such heterotic pools exist for sorghum, they have been developed for production environments in temperate and subtropical zones where advanced, highly mechanized agricultural techniques are used, such as in the USA and Australia [13–15].

A number of studies have investigated the utility of developing hybrids in sorghum for adoption in the semi-arid tropics of Africa [16,17] and in Ethiopia [4,18], based on combinations from introduced restorer (R) and male sterile (A) lines. These studies consistently identified hybrids that produced more grain yield than the parental lines and local check varieties; however, the hybrids lacked the adaptive traits for diverse local environments, were short in plant stature and had lower grain size. The development of heterotic pools adapted to a particular environment is one solution to overcome the challenges of both local adaptation and local farmers' end use requirements [19–21]. However such a strategy is complicated by the constraint that the cytoplasmic male sterile system imposes on developing new sorghum female parental lines [22]. In a recent study the genetic patterns of differentiation of locally adapted Ethiopian genotypes, in comparison to the introduced R and B lines, were identified using genome-wide SNP markers [23]. The current study focused on investigating the effectiveness of developing high yielding hybrids that also address the adaptation issue and multiple trait demands of farmers using selected locally adapted genotypes in combination with existing introduced seed parents and assessing whether there were correlations between parental genetic distance, using genome-wide SNP data generated previously, and performance. The specific aims of the current study were to 1) assess the performance of hybrids derived from locally adapted genotypes and introduced R lines in combination with introduced A-lines in contrasting environments in Ethiopia; and 2) assess the magnitude of heterosis within and between locally adapted and non-adapted genotypes.

2. Material and methods

2.1. Genetic materials

A total of 26 sorghum inbred lines consisting of 18 pollinator (R) and eight cytoplasmic male sterile (CMS) seed parental (A) lines were used to develop 139 F_1 hybrids using an unbalanced design II mating scheme (Table S1). These lines were selected from distinct groups identified among a diverse set of 184 Ethiopian genotypes selected from the Ethiopian working collection representing the highland, intermediate and lowland agro-ecologies, and introduced inbreds, differentiated using 11,788 genome-wide SNPs generated following integrated DArT and genotyping-by-sequencing (GBS) [23]. The genotyping method involved removal of repetitive sequences of genomic DNA through a complexity reduction process using methylation-sensitive restriction enzymes prior to sequencing on a Next Generation sequencing platform (DArT, http://www.diversityarrays.com/). The male parents included nine local Ethiopian genotypes and nine introduced R-lines (Table 1) selected based on agro-ecological adaptation and genetic distance of each group with the seed parent and within groups. The introduced R lines also consisted of early and medium maturity types with flowering dates between 58 and 75 days. In the absence of locally adapted female parental lines, the CMS lines selected included six A-lines introduced from Purdue University and ICRISAT and two Australian A-lines, which were bred for adaptation to low moisture environments. Crossing and seed multiplication of the F_1 hybrids were conducted in 2012 at an off season nursery at Melkawerer under irrigated conditions.

2.2. Description of test environments

Sorghum growing environments in Ethiopia can be broadly classified based on altitude, rainfall and length of growing period, identifying three major agro-ecological groups: highland (> 1900 m.a.s.l.), intermediate (1600–1900 m.a.s.l.) and lowland (< 1600 m.a.s.l.) [3,24]. Trials were grown at three research stations representing the three agro-ecologies in use by the National Sorghum Improvement Program in Ethiopia (Fig. S1). Arsi Negele (1960 m.a.s.l., 7°20′ N latitude) represented the highland environment, with a mean annual rainfall (RF) of 870 mm, minimum temperature (T_{min}) of 12 °C and T_{max} 25 °C, growing period of 180–200 days, and a vertisol soil type. The intermediate environment was represented by Bako (1565 m.a.s.l., 9°08′ N), with a mean annual rainfall of 1178 mm, T_{min} of 14 °C and T_{max} of 28 °C, growing period of 150–180 days, and a netosol soil type. The lowland environment was represented by Mieso (1470 m.a.s.l., 9°14′ N), with 713 mm mean annual rainfall, T_{min} of 14 °C and T_{max} of 34 °C, growing period of 110–120 days, and a vertisol soil type.

The average rainfall from 2006 to 2013 at the lowland site was half that received at the highland site. The lowland site also had an average 3 °C higher temperature in comparison to the other two contrasting environments. The highland environment was characterized by intermediate levels of rainfall and slightly lower temperatures during the early establishment and grain filling stages (Fig. S2). In comparison to the five years average, the amount of rainfall for the cropping season was similar to the average for the lowland testing environment, but higher for the highland and intermediate testing environments (Fig. S2).

2.3. Field trial setup

Field trials were conducted in the 2013 cropping season (June–November) in the three testing sites representing the contrasting major agro-ecologies in Ethiopia (Fig. S1). From the total of 139 F_1 hybrids generated, a subset of 93 was phenotyped in the lowland environment and 96 hybrids were tested in both the highland and intermediate environments (Table S1). Among the total number of testcross hybrids 48 were evaluated across the three environments; 66 were tested in the lowland and intermediate; 50 were tested in the lowland

Table 1 – Hybrid parental lines from locally adapted sorghum genotypes and introductions from ICRISAT, Purdue University and Australia.

Genotype name	Genotype group	Plant height	Race	Source
2005 MI 5065	Lowland adapted	200–250	Caudatum	Local improved
Misikir	Lowland adapted	150–200	Caudatum	Local improved
Ajab Sedi	Lowland adapted	200–250	Caudatum	Local landrace
Gambella 1107	Lowland adapted	150–200	Caudatum	Local landrace
PGRC/E 69475	Lowland adapted	200–250	Caudatum	Local landrace
PGRC/E 69241	Highland adapted	200–250	Durra	Local landrace
Wello Coll # 050	Highland adapted	> 250	Durra	Local landrace
Zengada 2	Highland adapted	> 250	Bicolor	Local landrace
2002 BK 7020	Intermediate adapted	150–200	Kafir	Local improved
ICSR 24004	Introduced R (early)	100–150	DC	ICRISAT
IESV 92031 DL	Introduced R (early)	150–200	Caudatum	ICRISAT
P 89009	Introduced R (early)	100–150	Caudatum	Purdue
PDL 984953	Introduced R (early)	100–150	NA	Purdue
PRL 984084	Introduced R (early)	100–150	NA	Purdue
PI 308453	Introduced R (medium)	150–200	NA	ICRISAT
104 GRD	Introduced R (medium)	200–250	NA	ICRISAT
E 237	Introduced R (medium)	150–200	NA	ICRISAT
ICSV 700	Introduced R (medium)	> 250	Durra	ICRISAT
A BON 34	Introduced B	100–150	NA	Purdue
ICSB 10	Introduced B	100–150	NA	ICRISAT
ICSB 21	Introduced B	100–150	NA	ICRISAT
ICSB 34	Introduced B	100–150	NA	ICRISAT
P 9517 B	Introduced B	100–150	NA	Purdue
P 9529 B	Introduced B	100–150	NA	Purdue
A 010054	Introduced B	100–150	NA	Australia
A 963676	Introduced B	100–150	NA	Australia

NA, not available; DC, Durra caudatum.

and highland environments; and 79 were tested in the highland and intermediate environments. Controlled randomization was based on grouping the inbred parents and test hybrids into three groups. The first group contained genotypes with plant heights <150 cm, the second group comprised genotypes between 150 and 200 cm, and the third group included genotypes >200 cm. The field trials were laid out using a triple lattice design for a total of 121 genotypes and included the hybrids, both male and female parents, and three checks. The first hybrid released in Ethiopia, using introduced inbred parents (P-9501 A × ICSR 14), and open pollinated improved varieties WSV 387 and 87 BK 4122 were used as checks in both environments.

The hybrids were planted following the recommended row spacing and fertilizer was applied for each test site because of an intended use of improved management practices to increase sorghum production. Each plot consisted of two 3.00 m rows, with 0.75 m inter-row spacing in all three environments (plot area, 4.5 m^2) and intra-row spacing of 0.20 m in lowland and 0.25 m in highland and intermediate environments. Based on the onset of rainfall, planting occurred at different dates across trials; July 15 in the lowland environment; June 19 in the intermediate environment; and May 18 in the highland environment. Fertilizer was applied at the rate of 100 kg ha^{-1} diamonium phosphate (DAP) and 50 kg ha^{-1} urea in the lowland environment, whereas in the highland and intermediate environments DAP and urea fertilizers were each applied at 100 kg ha^{-1}. DAP was applied during planting and urea was side dressed at the knee height stage in all environments.

2.4. *Data recording and statistical analysis*

For data collection five random plants from each plot were tagged and covered with paper bags after seed set to protect from bird damage and data were measured on each plant. Grain yield (GY) was measured based on the average head yield after adjusting to 11% moisture content and converted into kg ha^{-1} on the basis of the total plant populations for each test environment; plant height (PTH) was measured from the base to the top of each selected plant (cm); days to flowering (DTF) was measured as the date when 50% of the plants in plots had flowered and days to maturity (DTM) was measured as days from planting to grain maturity as indicated by black layers at the seed bases. One hundred grain weight (HGW) was measured as the weight of 100 seeds (g) sampled from the five harvested plants. The grain number (GN) per head was calculated based on the average grain yield/panicle (HY) of five randomly selected plants and hundred grain weight (HGW) using the formula [GN = (HY/HGW) × 100]. In addition, the daily rainfall and minimum and maximum temperatures for the growing periods were collated from weather stations located within 200 m from each research site.

Data were analysed using a linear mixed model with spatial variation accounted for by considering rows and columns as a covariate to estimate the variance components and best linear unbiased predictor (BLUP) of the genetic values for the traits measured using Genstat statistical software [25]. The predicted mean values were estimated considering entries as fixed effects and replications as random effects. The predicted hybrid performances were used to differentiate the different groups of genotypes for each test environment. The magnitude of heterosis of each hybrid and hybrid group, excluding the hybrids derived from intermediate adapted genotypes because of their limited number, were computed using predicted mean values. Heterosis was calculated as 1) mid-parent heterosis {$MPH = [(F_1 - MP)/MP] \times 100$}; and 2) better parent heterosis {$BPH = [(F_1 - BP)/BP] \times 100$}, where F_1 is the predicted mean performance of the hybrid, MP, is the average of the predicted performance of the two inbred parents, and BP is the predicted mean values for the better performing inbred parent. The significance of the mean performance of the test crossed hybrids over the better parent was tested using *t*-test values computed using: $t_{ij} = F_{1ij} - BP_{ij} / (\sqrt{\frac{1}{2}}EMS)$ [26], where F_{1ij} = mean of the *ij*th F_1 cross, BP_{ij} = best parent of the *ij*th cross, and EMS = error mean square for each hybrid group. In addition, standard heterosis (SH) was computed for each hybrid and group relative to the performance of the check variety and hybrid.

3. Results

3.1. *Genetic variability and hybrid performance in the three test environments*

Genetic variability for genotypes, hybrids and inbreds was significant ($P < 0.001$) in each environment for grain yield, days to flowering, plant height, grain number and hundred grain weight (Table S2). Overall, 60% of the total genetic variance for grain yield was explained by the genetic main effect while the remaining variance was accounted for by environmental and genotype × environment interaction effects. The overall mean hybrid grain yield performance was higher in the lowland than in the highland environments (6081 vs. 5029 kg ha^{-1}, respectively), and the lowest mean grain yield was obtained in the intermediate environment (4485 kg ha^{-1}).

There was significant correlation between highland and intermediate environments for grain yield performance (r = 0.66, $P < 0.000$), in addition to plant height (r = 0.95, $P < 0.000$), grain number (r = 0.69, $P < 0.000$), and HGW (r = 0.44, $P < 0.01$). In contrast significant positive correlations were obtained for plant height, days to flowering and grain weight between lowland and intermediate environments with the correlation coefficients ranging from 0.34 to 0.94.

3.2. *Hybrid performance and magnitude of heterosis for different groups of hybrids*

Mean hybrid performance and better parent heterosis (BPH) are detailed in Table 2 for the two groups of hybrids derived from Ethiopian genotypes adapted to the lowland (LLA) and highland (HLA) environments, and the third group of hybrids involving introduced R lines (IRL) and introduced A lines. Hybrids flowered and matured earlier in all test environments, but showed superior performance for the majority of measured traits, in comparison to the better parent. Variation between the hybrids and respective high parents was significant for grain yield for the three hybrid groups across the agro-ecologies (Table 2). However,

Table 2 – Predicted mean sorghum hybrid performance and mean better parent heterosis (BPH %) for grain yield (GY), days to flowering (DTF), days to maturity (DTM), plant height (PTH), internode length (Int length), internode number (Int number), grain number per head (GN), and hundred grain weight (HGW) for three groups of genotypes evaluated in lowland, highland and intermediate environments in Ethiopia.

Hybrid group		Lowland environment				Highland environment				Intermediate environment			
		LLA	HLA	IRL	SE	LLA	HLA	IRL	SE	LLA	HLA	IRL	SE
	Test cross	27	14	37		24	18	25		24	18	35	
GY (kg ha^{-1})	Mean	7223	6752	5598	541.5	5165	6703	4147	531.4	4553	5889	3878	514
	BPH (%)	19^{***}	42^{***}	23^{***}		43^{***}	16^{***}	33^{***}		19^{***}	52^{***}	10^{***}	
DTF	Mean	66	70	66	1.4	80	92	80	2.9	85	87	85	1.8
	BPH (%)	-9^{***}	-6^{***}	-8^{**}		-6^{**}	-11^{***}	-12^{*}		−1	−3	−2	
DTM	Mean	108	111	108	1.9	162	174	160	3.2	150	163	154	1.6
	BPH (%)	-4^{*}	-5^{***}	-4^{*}		−4	−4	−4		−5	−1	−4	
PTH (cm)	Mean	235	277	181	11.4	176	220	124	13.2	198	225	162	6.7
	BPH (%)	12	−3	16^{*}		14	-20^{**}	8		6	-13^{**}	15	
Int length	Mean	25.7	29.2	16.4	1.69	21.4	24.0	15.0					
	BPH (%)	9.7	37.8	23.0		13.7^{*}	−7	17.6					
Int number	Mean	8.1	9	6.9	0.71	6.4	7.8	5.6					
	BPH (%)	−2.4	2.1	1.1		−3.1	−16.8	−3.4					
GN head^{-1}	Mean	4033	3918	3550	541	3016	3507	2688	365.3	2332	3375	2237	306.4
	BPH (%)	17	43	19		44	−6	38		8	30	−2	
HGW (g)	Mean	2.6	2.6	2.3	0.2	3.0	3.3	3.1	0.27	2.7	2.7	2.7	0.13
	BPH (%)	1	2	3		−7	2	−4		1	10	4	

*, **, ***, Significant at $P < 0.05$, 0.01, and 0.001, respectively; SE, standard error.

there was no significant variation between the hybrids and the high parent in grain number and hundred grain weight (Table 2). Compared to the IRL hybrids, the LLA and HLA hybrids had grain yield advantages of 29% and 62% in their adaptation environments, respectively, in addition to increased plant height and grain weight. In comparison to the introduced seed parents (B lines) the locally adapted genotypes and 75% of the introduced R lines had the highest mean phenotypic values for all the traits measured in each environment (data not shown).

3.3. *Hybrid performance and better parent heterosis in the lowland environment*

LLA hybrids had a mean grain yield of 7223 kg ha^{-1} and mean BPH of 19% in the lowland environment (Table 2). Overall the majority (89%) of LLA hybrids had superior grain yield, ranging from 2% to 60% in comparison with the better parent (Fig. 1). In addition, the mean plant height of LLA hybrids was 235 cm, ranging from 179 to 254 cm. The mean BPH for plant height was 11%, with 56% of them being taller than the best parent. In addition, internode length had a mean BPH of 9.7% while the BPH for the internode number was −2.4%. The LLA hybrids flowered eight days earlier than their parents, with an average BPH of −9%. The mean BPH for grain number was 17%, with a large proportion (81%) of LLA hybrids having higher grain numbers relative to the best parent. Overall, half of the LLA hybrids had increased grain weight, with a mean BPH of 1% and maximum value of 24%. Nine (33%) of the LLA hybrids were superior to the best parent in both grain weight and grain number.

In comparison to the HLA and IRL hybrids, LLA hybrids had the highest mean grain yield and grain number. In addition, the mean plant height and grain weight were higher for LLA hybrids in comparison to IRL hybrids. The grain yield advantage of LLA hybrids in comparison to HLA and IRL hybrids was, on average, 7% and 29%, respectively. However, six of the HLA hybrids had comparable grain yield performances to the LLA hybrids in the lowland environment. Two of these HLA hybrids had higher grain yields compared to the high yielding LLA hybrid (ICSA 34 × PGRC/E 69475). However, the *per se* performance of the highland adapted parents was 17% lower than the lowland adapted parent (data not shown). This was reflected in the magnitude of grain yield BPH (42%) for HLA hybrids, which was higher than LLA and IRL hybrids grown in the lowland environment (Table 2, Fig. 2-A). The average mean BPH for days to flowering for HLA hybrids was −12%, with flowering occurring eight days earlier than the best parent; however, these hybrids flowered an average four days later than the LLA and IRL hybrids. The mean days to maturity were 108 days for LLA and IRL hybrids, ranging from 103 to 113 days. In comparison, the HLA hybrids had mean days to maturity of 111 days, ranging between 105 and 120 days.

3.4. *Hybrid performance and better parent heterosis in the highland environment*

The mean grain yield for HLA hybrids was 6703 kg ha^{-1} with 16% superiority for the best parent (Table 2). In addition, 61% of the hybrids had superior grain yield performance, ranging from 2.5% to 73.0% yield increases in comparison to locally adapted best parental lines (Fig. 3-A). HLA hybrids had positive BPH for grain weight (2%) but negative BPH for grain number (−6%). However, variation was observed in the magnitude of BPH for grain number and grain weight among HLA hybrids. Hybrids derived from durra type parental lines, PGRC/E69241 and Wello coll #050, had a negative mean BPH (−17%) for grain number and a positive mean BPH (4%) for grain weight, whereas hybrids derived from the bicolor parental line, Zengada 2, had a positive mean BPH (21%) for grain number and a negative mean BPH (−2%) for grain weight.

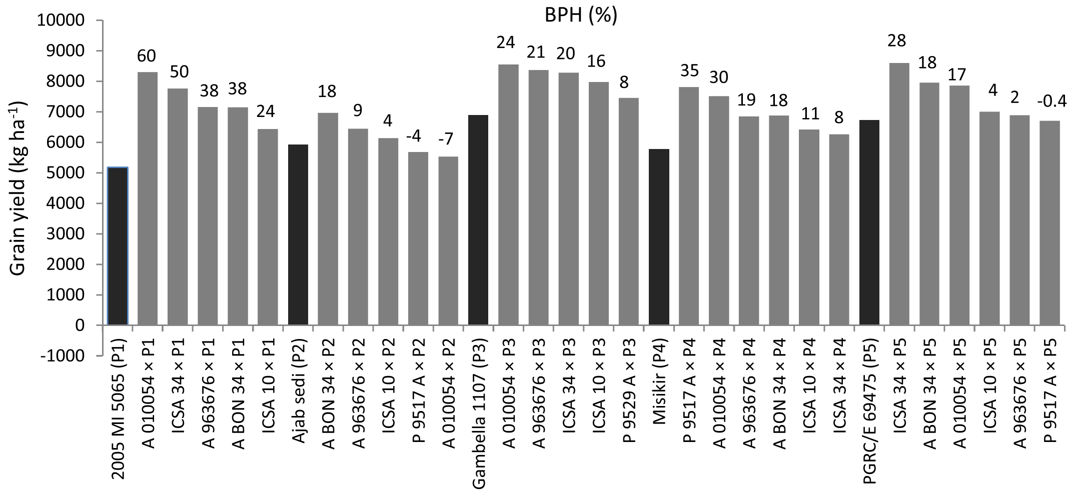

Fig. 1 – Sorghum hybrid grain yield performance (kg ha^{-1}), better parent heterosis (BPH) and standard error bar (horizontal line) of LLA hybrids and high yielding parents evaluated in the lowland environment color coded as follows: lowland adapted hybrid parent (dark) and the hybrids crossed with different female parents (grey).

Additionally, all HLA hybrids flowered earlier than the inbred parent at an average BPH of −11% and also exhibited a negative mean BPH for plant height (−20%). The HLA hybrids had a −7% mean BPH for internode length and −18% BPH for internode number.

Although HLA hybrids gave the highest mean performance for all five traits measured in comparison to LLA and IRL hybrids, the highest BPH for grain yield was obtained for LLA hybrids (43%) followed by IRL hybrids (33%) (Table 2, Fig. 2-B). However low *per se* performance for the inbred parent of the LLA and IRL hybrids was observed compared to the highland adapted genotypes, which had a twofold higher mean grain yield than the two groups of inbreds (data not shown). In the lowland environment, three hybrids derived from the non-adapted highland-adapted and introduced R lines, and in the highland environment two hybrids derived from introduced R and lowland-adapted genotypes exhibited outlier HPH (Fig. 2). The LLA and IRL hybrids flowered an average 12 days earlier and had lower grain numbers and grain weights than the HLA hybrids. However, both the LLA and IRL hybrids exhibited positive BPH for grain number, with the highest value of 44% for the LLA hybrids but negative BPH in grain weight (−7%).

3.5. *Hybrid performance and heterosis in the intermediate environment*

The performance and magnitude of heterosis for the three hybrid groups were also evaluated in the intermediate environment. For the HLA hybrids, the direction of BPH was the same as the highland environment for all traits except grain number. LLA and IRL hybrids had positive BPH for all traits, except grain number and days to flowering for the IRL hybrids and days to flowering for the LLA hybrids. The HLA hybrids had the highest mean BPH for grain yield (52%) in comparison to the other two groups of hybrids. The majority

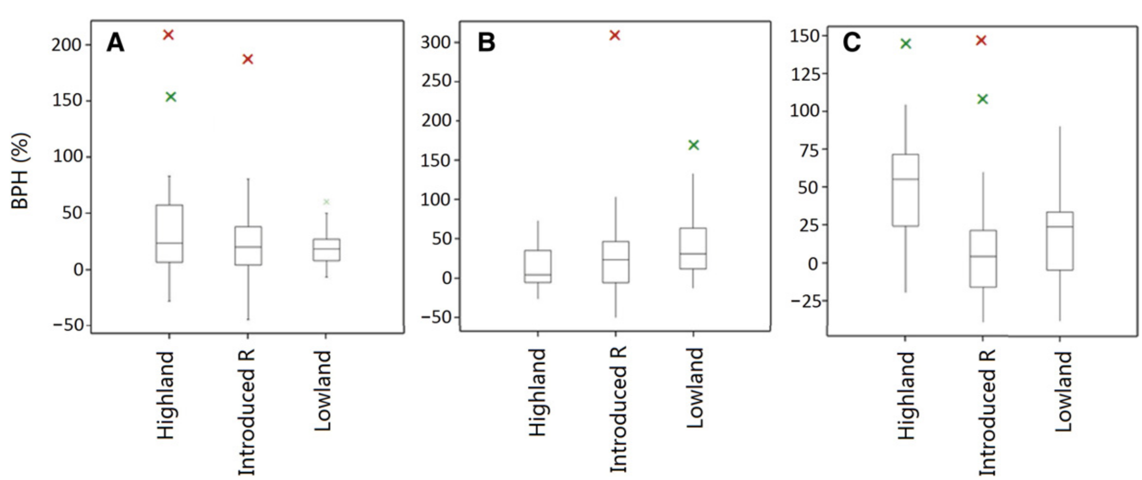

Fig. 2 – Box plots for grain yield better parent heterosis (BPH) of sorghum hybrids derived from Ethiopian genotypes adapted to highland (highland) and lowland environments (lowland) and introduced R lines (introduced R) evaluated in: A, lowland; B, highland; and C, intermediate environments.

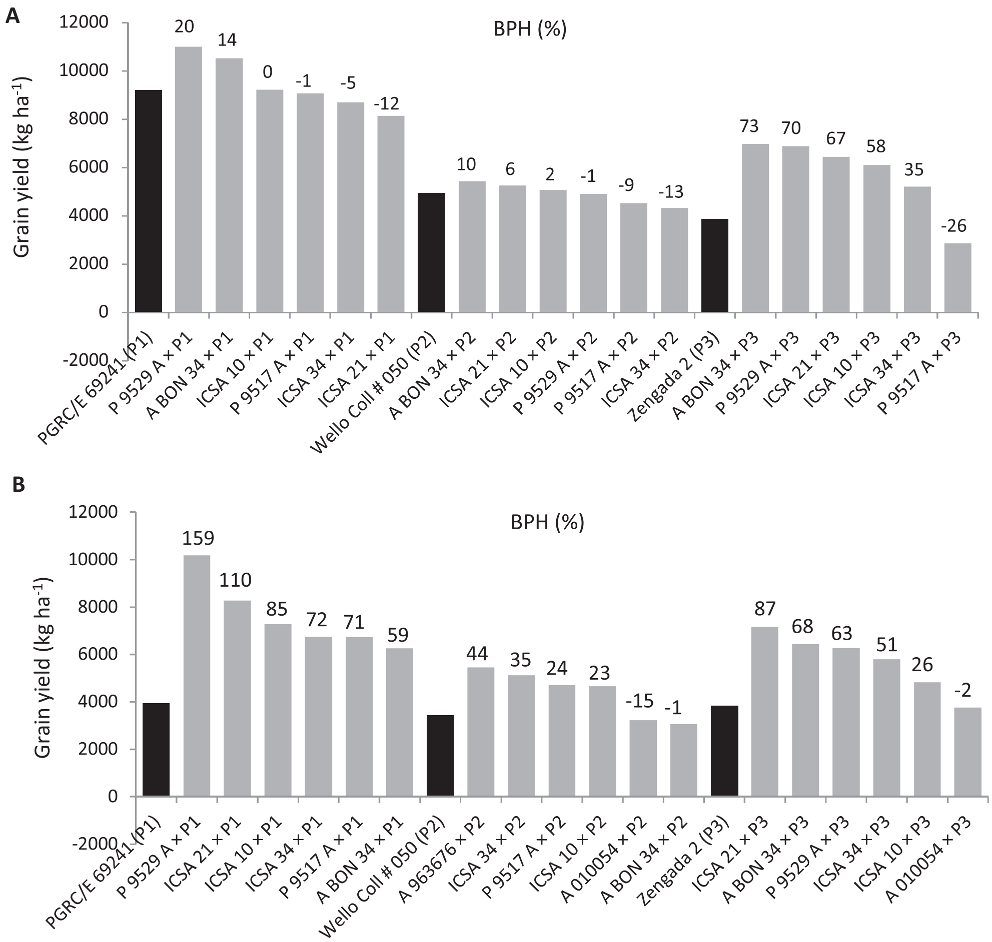

Fig. 3 – Sorghum hybrid grain yield performance (kg ha^{-1}) and better parent heterosis (BPH) of HLA hybrids and high yielding parents color coded as follows: highland adapted parent (black) and their hybrids crossed with different female parent (grey) evaluated in the: A. highland; and B. intermediate environments.

of HLA hybrids (83%) had positive BPH for grain yield ranging from 23% to 158% (Fig. 3-B). In comparison to the highland and lowland environments, the magnitude of BPH for grain yield for HLA hybrids was higher in the intermediate environment. LLA hybrids had similar mean BPH in both lowland and intermediate environments whereas IRL hybrids had the lowest BPH compared to the lowland and highland environments and were also lower than the HLA and LLA hybrids (Fig. 2-C).

3.6. *Genetic distance using SNP markers as a predictor of hybrid grain yield and better parent heterosis*

There was a weak positive correlation between inbred line genetic distance and hybrid grain yield performance and BPH in the lowland and highland environments. In the lowland environment, the magnitude of correlation between genetic distance and hybrid GY performance was (r = 0.13) whereas the correlation between BPH and genetic distance was (r = 0.17) for all hybrid groups. In the highland environment, genetic distance between inbred parental lines was significantly correlated (r = 0.25, P < 0.03) with hybrid GY performance, but there was no significant correlation between genetic distance and BPH for grain yield. However, in the intermediate environment, genetic distance between the inbred parents was significantly correlated with grain yield performance (r = 0.35, P < 0.001) and grain yield BPH (r = 0.4, P < 0.001). Genetic distance between the inbred parents and mid-parent heterosis (MPH) had an improved correlation (r = 0.51, P < 0.001) in the intermediate environment, whereas in both the lowland and highland environments improvement in prediction from HPH was not realised. Furthermore, the correlation coefficient was not improved by sub-grouping hybrids based on adaptation of the male parental lines to specific environments.

3.7. *Standard heterosis*

In the lowland environment, the improved open pollinated check variety Melkam had a mean grain yield of 6482 kg ha^{-1} while the recently released sorghum hybrid derived from introduced inbreds (P-9501 A × ICSR 14) had a mean grain yield of 5985 kg ha^{-1}. The standard heterosis (SH) for the hybrids was calculated using the higher yielding check variety is presented in Table 3. The LLA hybrids had an average SH of 11% from Melkam and 21% from the hybrid check. The majority (20 of 27) of the LLA hybrids had superior performance over the check variety. Although the mean SH of the IRL hybrids was negative, 13 of 37 IRL hybrids out-yielded the check variety. The highest SH for the HLA hybrids was 48%

Table 3 – Mean and maximum standard heterosis (SH) relative to the improved variety (Melkam) in the lowland and 87 BK 4122 in the intermediate and highland environments in Ethiopia.

Hybrid group	Standard heterosis (%)											
	GY (kg ha^{-1})		DTF		DTM		PTH (cm)		GN head^{-1}		HGW (g)	
	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.
Lowland environment												
Lowland adapted (LLA)	11	33	−10	−15	−5	−8	44	75	8	36	−2	26
Highland (HLA)	4	48	−4	−14	−2	−7	70	92	5	43	−1	21
Introduced R (IRL)	−14	13	−10	−21	−5	−9	11	60	−4	31	−11	6
Highland environment												
Lowland adapted (LLA)	4	58	−8	23	−4	−7	65	106	−31	8	5	32
Highland adapted (HLA)	35	121	5	26	−1	−6	105	148	−20	36	16	46
Introduced R (IRL)	−17	17	−7	−17	−4	−6	16	82	−39	−10	10	32
Intermediate environment												
Lowland adapted (LLA)	−20	34	5	10	−0.6	−3	35	54	−21	61	2.1	21
Highland adapted (HLA)	3	68	7	15	−0.5	−5	54	89	15	87	1.3	21
Introduced R (IRL)	−32	1	5	10	−0.9	−3	11	49	−24	10	1.1	19

and for LLA hybrids 33% compared to the check variety. The increase in plant height was 70% for the HLA and 44% for the LLA hybrids in comparison to the check variety. The average SH for grain weight was negative for both hybrid groups; however, 12 LLA and 8 HLA hybrids had higher grain weight than the check variety (Table 3). On average both the LLA and IRL hybrids flowered and matured earlier than the check variety but were later than the check hybrid.

HLA hybrids had the highest grain yield with a mean SH of 35% in the highland and 3% in the intermediate environment in comparison to the check variety, 87 BK 4122 (Table 3). The HLA hybrids flowered late and had an average SH of 105% for plant height and 16% for grain weight in the highland environment in comparison to the check variety, and showed increases in both traits in the intermediate environment. In the highlands, the LLA hybrids had 4% mean grain yield superiority in addition to increased plant height of 65% and grain weight by 5% in comparison to the check variety. Except for plant height and grain weight, the IRL hybrids had lower performance for other traits relative to the check variety in the highland and intermediate environments (Table 3).

4. Discussion

This study demonstrated that hybrids derived from Ethiopian genotypes adapted to lowland and highland environments, in combination with introduced A-lines, exhibited higher mean grain yields in their respective adaptive environments compared to the high parent and the hybrids derived from introduced R lines. The results indicated the potential of hybrids derived from locally adapted genotypes to increase sorghum productivity while meeting increased plant height and grain size requirements of sorghum-growing farmers in Ethiopia. Highland adapted hybrids (HLA) had the highest mean grain yield better parent heterosis (BPH, 52%) in the intermediate environment; BPH was also highest in comparison to lowland adapted hybrids (LLA) and introduced R lines hybrids (IRL). The variation in the magnitude of heterosis likely reflects the increased genetic distance between the highland adapted parental lines and introduced A-lines in comparison to the other two groups of genotypes [23]. In addition, compared to the standard check the high yielding LLA hybrids had 33% and HLA hybrids 121% grain yield superiority in their adaptive environments, indicating the potential to increase sorghum productivity in Ethiopia using hybrids developed using locally adapted genotypes as inbred parents.

4.1. *Superior performance of hybrids from locally adapted inbred parents*

The mean BPH for grain yield in each environment using locally adapted and non-adapted hybrid groups ranged between 10% and 52% (Table 2). This is within the range previously reported for sorghum in the USA [9,27] and sub-Saharan Africa [10,17]. Hybrids derived from locally adapted genotypes had the highest mean hybrid performance compared to the non-adapted parental lines in their adaptive environment. In the lowland environment, LLA hybrids had a yield advantage of 7% in comparison to the HLA hybrids and 29% in comparison to IRL hybrids. In addition, HLA hybrids had 30% yield superiority compared to LLA hybrids and 62% superiority compared to IRL hybrids in the highland environment (Table 2); however, the small sample size of highland-adapted hybrids may have contributed to magnitude of their superior performance. Previous studies also found superior phenotypic performances of hybrids derived from genotypes specifically adapted to their respective environments [19,28].

Drought stress is the critical limiting factor for sorghum production in lowland environments in Ethiopia [29]. Hence, drought tolerant, high yielding, tall sorghum varieties are preferred by farmers [4,7]. In this study, LLA hybrids exhibited a mean BPH of 19%, with a maximum value of 60%, in grain yield performance from the best yielding parent in the lowland environment. LLA hybrids had negative mean BPH for days to flowering (−9%) and for days to maturity (−4%), indicating earlier maturity of hybrids compared to their parents. However, plant height increased in LLA hybrids by 11% relative to the high parent, an important attribute for increased plant biomass [30,31]. Six of the HLA hybrids also had comparable grain yield performance to LLA hybrids in the lowland trials; however, these hybrids were later maturing by four days indicating a higher risk of terminal moisture stress in the lowland environment.

In addition to increased yield and plant height, larger grain size varieties attract higher acceptance by consumers and bring better incomes to farmers [4]; larger grain size is related to increased milling yield and higher water absorbance [5]. Half of the LLA hybrids had higher grain weight, of which nine also had higher grain number compared to the high parent demonstrating the possibility of improving grain yield and grain weight without sacrificing grain number when using locally adapted genotypes [32]. For instance the hybrid ICSA 10 × Gambella 1107, among the top yielding hybrids, had a 5% increase in grain number and 10% increase in grain weight compared to the high parent in the lowland environment.

The HLA hybrids had increased grain yield productivity with an average BPH of 16% in the highland and 52% in the intermediate environment. The latter was higher than the 26% yield increase recently reported in hybrids generated from guinea type sorghum in West Africa [21]. In relation to the higher grain number and grain weight BPH, the magnitude of grain yield heterosis for the HLA hybrids was higher in the intermediate than in the highland environment (Table 2). As the seed parents used for hybrid development were not bred for cold tolerance, lower temperatures in the highlands compared to the lowlands during establishment, might have had an effect on hybrid vigor in the highland environment (Fig. S2). The grain yield performance was significantly correlated ($r = 0.66$, $P < 0.000$) between the highland and intermediate environments, likely explaining the similarity in ranking of the hybrids and the potential of hybrids in both environments. HLA hybrids, however, had negative BPH for plant height in all three environments. This was possibly due to reduced internode length related to incomplete dominance of the plant height genes [33]. In order to overcome a reduction in plant height and grain number, a strategy for breeding locally adapted male sterile female parents with increased plant height and cold tolerance could be employed.

4.2. Hybrid performance and heterosis of locally adapted genotypes versus introduced R lines

The male and female parental groups currently being used for hybrid breeding were primarily developed for adaptation to temperate environments where mechanized farming and high input systems are in place [14,15,34]. In addition to the superiority in grain yield performance, adaptation of the hybrids to local environments and improvement of farmers' preferred traits, including plant height and grain size, are vital for hybrids to be adopted by Ethiopian farmers [4,7]. In this study the LLA and HLA hybrids gave 29% and 62% increases in grain yield, respectively, in addition to 30% and 77% increased plant height compared to IRL hybrids in their adaptive environments indicating the possibility of developing adaptable, high yielding hybrids using locally adapted genotypes [19,28]. Although LLA and IRL hybrids had similar flowering and maturity times LLA hybrids had increased grain yield, plant height, grain number and grain weight in comparison to IRL hybrids. Correlation between increased grain yield and plant height in sorghum hybrids was reported in previous studies [35], and may be related to higher radiation use efficiency by tall, locally adapted sorghum hybrids. Variation in grain number and grain size between LLA and IRL hybrids was also observed, with the LLA hybrids having larger grains relative to IRL hybrids (2.6 g vs. 2.3 g 100 seed^{-1}). This again could be related to the availability of assimilate in the tall locally adapted hybrids during late grain filling stages, as observed in previous studies [35–36].

The success of hybrid breeding depends on the magnitude of heterosis for economically important traits and this is related to the extent of genetic variability and complementarity of parental pools [37]. The existing restorer and seed parent groups in sorghum impose genetic constraints due to the complex cytoplasmic male sterility system, which limits the development of new seed parents [22]. However, there is potential to expand the pollinator pools due to the predominance of restorer genes in a range of genetic backgrounds of sorghum genotypes [22]. The magnitude of better parent heterosis for hybrids derived from locally adapted genotypes suggests that it will be possible to exploit heterosis in Ethiopia using the existing seed parents across all environmental types with the exception of the highlands, due to the lack of cold temperature adaptation in the existing seed parents. Although there is variation in BPH depending on adaptation of the inbred lines, the extent of BPH of LLA and IRL hybrids seemed to be within the same range in the lowland and highland environments (Fig. 2-A,B).

5. Conclusion

In this preliminary study hybrids developed using locally adapted genotypes as a restorer line crossed with introduced A-lines exhibited higher grain yield performance than the high parent and the hybrids derived from introduced R-lines. The magnitude of grain yield superiority over the high parent extended up to 60% and 73% for the LLA and HLA hybrids in the lowland and highland environment, respectively. This result highlights the potential to increase sorghum productivity while increasing plant height and grain size using locally adapted genotypes as hybrid parents. The superiority of the test hybrids over the check variety included in this study indicates the potential positive economic advantage of such hybrids in the diverse sorghum-growing environments in Ethiopia. Breeding A/B lines adaptable to the highland environment or selection for cold tolerance among highland-adapted genotypes could possibly overcome the shortcomings of HLA hybrids. The magnitude of better parent heterosis was higher for HLA hybrids compared to IRL and LLA hybrids and corresponded with the genetic distance separating the inbred parental lines. This result might be an indication of the distinct heterotic grouping of highland-adapted landrace genotypes, but that aspect needs further verification.

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Supplementary data

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