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RESEARCH

Improving Maize Grain Yield under Drought Stress and Non-stress Environments in Sub-Saharan Africa using Marker-Assisted Recurrent Selection

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ABSTRACT

In marker-assisted recurrent selection (MARS), a subset of molecular markers significantly associated with target traits of interest are used to predict the breeding value of individual plants, followed by rapid recombination and selfing. This study estimated genetic gains in grain yield (GY) using MARS in 10 biparental tropical maize (*Zea may* L.) populations. In each population, 148 to 184 $F_{2:3}$ (defined as C_0) progenies were derived, crossed with a single-cross tester, and evaluated under water-stressed (WS) and well-watered (WW) environments in sub-Saharan Africa (SSA). The C_0 populations were genotyped with 190 to 225 single-nucleotide polymorphism (SNP) markers. A selection index based on marker data and phenotypic data was used for selecting the best C_0 families for recombination. Individual plants from selected families were genotyped using 55 to 87 SNPs tagging specific quantitative trait loci (QTL), and the best individuals from each cycle were either intercrossed (to form C_1) or selfed (to form C_1S_1 and C_1S_2). A genetic gain study was conducted using test crosses of lines from the different cycles F_1 and founder parents. Test crosses, along with five commercial hybrid checks were evaluated under four WS and four WW environments. The overall gain for GY using MARS across the 10 populations was 105 kg ha^{-1} yr^{-1} under WW and 51 kg ha^{-1} yr^{-1} under WS. Across WW environments, GY of C_1S_2–derived hybrids were 8.7, 5.9, and 16.2% significantly greater than those of C_0, founder parents, and commercial checks, respectively. Results demonstrate the potential of MARS for increasing genetic gain under both drought and optimum environments in SSA.

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Abbreviations: AD, anthesis date; ASI, anthesis–silking interval; GS, genomic selection; GY, grain yield; MABC, marker-assisted backcrossing; MARS, marker-assisted recurrent selection; MAS, marker-assisted selection; mQTL, meta-QTL; P-MTI, multiple-trait phenotypic index; PH, plant height; QC, quality control; QTL, quantitative trait loci; SD, silking date; SNP, single-nucleotide polymorphism; SSA, sub-Saharan Africa; WS, water-stressed; WW, well-watered.

Maize is one of the world's most important food crops. In SSA countries, it is the most important staple food for over 300 million people. However, GY are highly variable and low; for example, between 2011 and 2013, the average maize GY in SSA was estimated at 1.8 Mg ha^{-1} compared with 2.8 Mg ha^{-1} in the Philippines, 3.1 Mg ha^{-1} in Mexico, and 4.4 Mg ha^{-1} in Thailand (http://faostat3.fao.org). Although an array of factors contributes to this low productivity, low fertilizer use and drought are the major abiotic factors limiting maize production and productivity in SSA (Shiferaw et al., 2011). According to Heisey and Edmeades (1999), about 20 to 25% of the global maize production area is affected by

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drought in any given year. Drought tolerance is needed for farmers to achieve high and stable maize GY and for seed companies to be able to widely market a maize cultivar.

Maize is most susceptible to stress at flowering when silk growth, pollination, and kernel set occur (Shaw, 1977). Water stress slows ear growth, and consequently silk emergence, more than tassel growth or anthesis, resulting in a widening interval between ASI. Severe stress at flowering may lead to the complete abortion of ears and the plant becomes barren. Drought-affected ears typically have fewer kernels that will be poorly filled if drought extends throughout grain filling (Edmeades et al., 2000). Yield, under stress at flowering, shows a strong dependency on kernel number per plant ($r > 0.8$), bareness ($r > 0.7$), and ASI ($r = -0.4$ to -0.7) in tropical maize (Bolaños and Edmeades, 1996). The ASI becomes a reporter trait for ear and plant growth rates during the flowering period (Edmeades et al., 1993, 2000; Vega et al., 2001).

Improving drought tolerance is a key breeding objective of the CIMMYT maize breeding program. In the 1970s, CIMMYT initiated selection for drought tolerance in tropical maize by evaluating a large range of germplasm under drought stress at flowering, drought stress at grain-filling, and under WW environments (Edmeades et al., 1997). Selection gains in tropical maize were associated with increased flowering synchronization (i.e., a reduced ASI), fewer barren plants, smaller tassel size, a greater harvest index, and delayed leaf senescence (Bolaños and Edmeades 1993). Bänziger et al. (2006) compared hybrids developed using a CIMMYT-managed drought stress protocol with the commercial hybrids in southern Africa across 36 to 65 environments and reported that CIMMYT-selected hybrids produced a yield advantage of 13 to 20 and 3 to 6% in the low and high potential environments, respectively.

Molecular markers can be used to speed up the development and deployment of improved germplasm in different ways, including marker-assisted backcrossing (MABC), MARS, and genomic selection (GS). Marker-assisted backcrossing is the simplest form of marker-assisted selection (MAS) used to transfer one or few genes or major effect QTL that are fine mapped and validated across different genetic backgrounds. Several researchers (Veldboom and Lee 1996; Ribaut et al., 1997; Almeida et al., 2013; Semagn et al., 2013) have focused on mapping QTL associated with drought stress tolerance in different maize mapping populations. Using MABC, Ribaut and Ragot (2007) introgressed five QTL associated with yield components and flowering in maize from a donor parent into a drought-susceptible recurrent parent. The authors reported increased GY and reduced ASI under water-limited environments. However, the background specificity of the identified QTL and the absence of QTL with large individual effects have limited their applicability in maize improvement for drought tolerance (Tuberosa et al., 2007; Araus et al., 2008). Recently, Semagn et al. (2013) conducted meta-QTL (mQTL) analyses across 18 biparental tropical maize populations evaluated in two to four WS and three to four WW environments in Kenya, Zimbabwe, and Zambia. The meta-analyses reduced the number of QTL for GY and ASI from 183 to 68 and narrowed the confidence intervals up to 12-fold. Each mQTL explained, on average, between 1.2 and 13.1% of the phenotypic variance for GY and ASI, and the overall mean was 6.5%. Nine mQTL were detected in both environments and in up to six genetic backgrounds but none in more than six genetic backgrounds. The cross specificity of the identified QTL, the genotype × environment effects, and the absence of large-effect QTL have limited the applicability of the detected QTL in maize improvement using MABC.

To overcome some of the limitations associated with MABC, both MARS and GS have been used in several studies. Marker-assisted recurrent selection is a marker-based breeding method that seeks to accumulate favorable alleles from several genomic regions within a single population (Edwards and Johnson, 1994). In contrast to MABC, that targets genes or major-effect QTL that have been fine mapped and validated across different genetic backgrounds, MARS aims at accumulating relatively large number of medium-effect QTL in a given population using a subset of markers that are significantly associated with target traits (Bernardo, 2008). Every MARS population is handled independently, so QTL information generated in one population is not often transferable into another population.

Xu et al. (2012) proposed MARS as an effective tool to breed for complex traits because it enables harnessing even those genes or QTL exhibiting minor effects of the phenotype. Marker-assisted recurrent selection has been used to successfully enhance quantitative traits in maize (Johnson 2004; Massman et al., 2013). Eathington et al. (2007) compared MARS and conventional selection in 248 North American and European maize breeding populations, and reported higher performance and more than double gains for MARS than for phenotypic selection. They also found that the MARS-derived lines were higher performing than conventionally selected lines.

Genomic selection (Meuwissen et al., 2001) is another marker-based strategy that incorporates all available marker information simultaneously into a model to predict the genetic value of progenies for selection (Lorenz, 2013). Each marker is considered a putative QTL, reducing the risk of missing small-effect QTL (Guo et al., 2012). A recent study (Beyene et al., 2015) in maize in SSA using data of eight biparental populations evaluated under WS environments showed 13.4 and 18.9% greater mean GY for hybrids derived from Cycle 3 of GS than with C_0 and the best commercial checks, respectively. To our knowledge, however, there are no reports describing the use of MARS to improve tropical maize germplasm under WS and WW environments. The objective of this study was

Table 1. Summary of the 10 populations, including founder parents, number of single-nucleotide polymorphism (SNP) markers used for both mapping and marker-assisted recurrent selection (MARS), and heritability (*H*) for grain yield (GY) and anthesis silking interval (ASI) under water-stressed (WS) and well-watered (WW) environments in sub-Saharan Africa.

Population code	Parent 1†	Parent 2†	*H* for GY under WW	*H* for GY under WS	*H* for ASI under WS	$F_{2:3}$ population size	No. of SNPs used for genotyping $F_{2:3}$	No. of significant SNPs used for selection
6x1008	CML540	CML505	0.42	0.24	0.40	165	201	64
6x1015	CZL04003	CML540	0.35	0.26	0.21	162	190	62
6x1016	CML540	CZL99017	0.57	0.13	0.36	148	191	63
6x1017	CML540	CML539	0.47	0.19	0.41	184	210	70
6x1018	CML505	CZL99017	0.52	0.26	0.08	184	212	87
6x1019	CZL04008	CZL0719	0.38	0.03	0.33	173	202	63
6x1020	CML542	CZL0724	0.55	0.33	0.34	181	218	55
6x1021	CML542	CZL0719	0.58	0.20	0.45	184	217	79
6x1023	CZL0618	VL062655	0.38	0.29	0.51	184	225	72
6x1028	CZL074	VL062645	0.40	0.05	0.37	174	204	66

† All lines with CML (CIMMYT Maize Lines) as prefix have commercial value, while CZL and VL lines are drought-tolerant donors.

to estimate genetic gains in GY across different cycles of selection in 10 biparental MARS populations evaluated in four WS and four WW environments in SSA.

MATERIALS AND METHODS

Population Development, Phenotyping of $F_{2:3}$ Test Crosses, and Genotyping

As part of the Water Efficient Maize for Africa project, a total of 13 MARS populations were developed in 2009. Quality control (QC) genotyping of F_1 hybrids and their founder parents with 100 SNP markers distributed across all maize chromosomes identified 10 F_1 hybrids with true-to-type parental alleles for ≥95% of the polymorphic SNPs for advancement to $F_{2:3}$, while those with >5% nonparental alleles were discarded. Three of 13 MARS populations failed the QC criteria and were excluded from advancement through MARS. For each of 10 MARS populations that passed the QC genotyping criteria, DNA was extracted by bulking equal amount of leaf tissue from 15 $F_{2:3}$ plants per family and genotyped with 190 to 225 SNPs using the TaqMan assay (http://www.appliedbiosystems.com) at the Monsanto Company, Ankeny, IA, USA.

Table 1 provides a summary of the 10 MARS populations, including the founder parents of each population, heritability for GY and ASI under WS and WW environments, the $F_{2:3}$ (C_0) population size, and the number of SNP markers used for genotyping C_0 for mapping and selection. The various steps followed during the MARS process are summarized in Fig. 1. The 10 populations were derived from crosses between CIMMYT drought-tolerant donors and CIMMYT inbred lines currently in commercial use in eastern and southern Africa. Test crosses were generated by crossing C_0 families with a single-cross tester (CML395/CML444) from a complementary heterotic group. Test crosses of each population, together with five commercial checks (WH504, WH505, H513, CZH0616, and DK8033), were planted in α-lattice designs with two replications and phenotyped at two or three WS environments and three or four WW environments in Kenya, Zimbabwe, and Zambia in 2010 (Semagn et al., 2013).

The WS experiments were conducted during the dry (rain-free) season by suspending irrigation starting from 2 wk before flowering until harvest, whereas the WW experiments were conducted during the rainy season, applying supplemental irrigation as needed. Entries were evaluated for several agronomic traits, but only GY at 12.5% moisture content and ASI were used to compute the selection index (see below). Selection for reduced ASI in tropical open-pollinated varieties has been shown to be correlated with improved yields under drought stress (Bolaños and Edmeades 1996). Data were analyzed within the WW and WS environments using a linear mixed model that considered genotypes and experiments as fixed and random effects, respectively.

Selecting C_0 to Form C_1

Marker-assisted recurrent selection was conducted by intermating selected C_0 families to form Cycle 1 (C_1) and then selfing superior C_1 plants for two generations to form C_1S_1 and C_1S_2 (Table 2). Marker–trait regression analysis was performed for identifying SNPs that were significantly associated with GY and ASI using Monsanto's proprietary software and protocols. Grain yield and ASI were combined into a multiple-trait phenotypic index (P-MTI) using the following weight: 80% for GY under WS and WW and 20% for ASI under WS environments. The two traits were standardized within the WS and WW experiments to equalize their relative weights in the index. At each marker, the additive genetic effect associated with each parental allele was calculated. For each population, a significant marker loci (at $p = 0.10–0.15$) was selected and a marker score M-MTI was calculated for each individual as the sum of allelic effects across selected significant loci. Finally, for each individual, we combined P-MTI and M-MTI values into a phenogenotypic index (I-MTI) as described in a Lande–Thompson selection index (Lande and Thompson 1990) that combined phenotypic and marker data. Based on marker-trait analysis, 55 to 87 SNPs (Table 1) were selected that had a significant effect on the selection index.

For each population, C_0 families were ranked based on Lande–Thompson selection index (Lande and Thompson 1990) and the top eight families were selected for planting. Two weeks

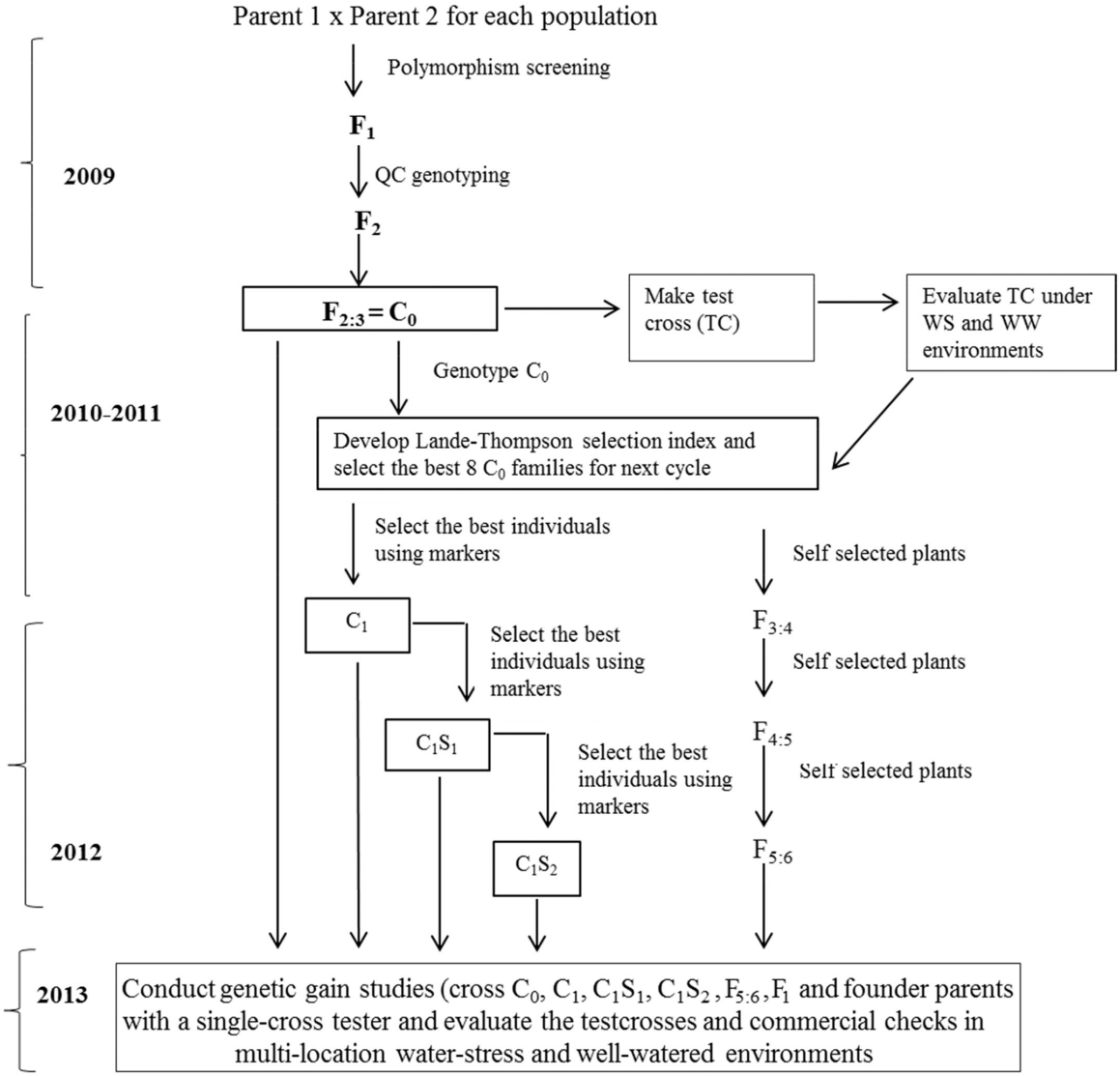

Figure 1. Summary of the various steps followed in developing improved maize germplasm using marker-assisted recurrent selection (MARS) and pedigree selection.

Table 2. Number of families and individual plants planted, selected, and advanced to the next MARS cycle.

Cycle	Form C_1 from C_0	Form C_1S_1 from C_1	Form C_1S_2 from C_1S_1
Total no. of individuals per population	88	184	184
No. of families	8	8	23
No. of individuals per family	11	23	8
No. of crosses or selfing	24	40	92
Crossing operation	recombine	self	self
No. of individuals or families selected for next cycle	8	23	92
No. of nursery rows	8	8	23

after planting, four leaf discs from each of 11 individuals per C_0 family were collected from the field, lyophilized at the Biosciences for Eastern and Central Africa Laboratory in Nairobi, Kenya (Semagn, 2014), and shipped to the Monsanto Company. Each individual was genotyped with the subset of 55 to 87 SNPs (Table 1) that were selected from the marker-trait analyses described above. Within each population, a total of 24 individuals (at least one from each selected family) were selected based on favorable allele frequency for GY and ASI. Each selected plant was used once as a female and from four to six times as a male; up to four alternative males were identified to obtain sufficient pollen for pollinating each female parent. Each female plant was pollinated by one of the selected males depending on pollen synchronization. At harvest, the top eight successful crosses (Table 2) were selected based on favorable allele frequency and seed set. Selected ears were advanced for the next planting cycle (C_1).

Marker Based Selection in C_1 and C_1S_1

For each population, the eight selected C_1 families were planted ear-to-row. Two weeks after planting, a total of 184 plants (each family represented by 23 C_1 plants) were genotyped with 55 to 87 SNPs at the Monsanto Company genotyping lab as described above. Based on genotypic information, the best 40 C_1 plants, with at least one plant selected from each C_1 family (to keep

segregation for the non-selected regions), were self-pollinated to form C_1S_1. At harvest, the best 23 C_1S_1 families were selected based on favorable allele frequency and seed set. For each population, the 23 selected C_1S_1 families were planted ear-to-row. Two weeks after planting, a total of 184 plants (each family represented by eight plants) were genotyped with 55 to 87 SNPs at the Monsanto genotyping lab; based on genotypic data, the best 92 plants (at least one plant per family) were selected and self-pollinated to form the C_1S_2. In each population, 92 C_1S_2 ears were harvested and shelled individually. Family structure was maintained at all steps of the MARS protocol. The selected population sizes for each generation, the number of selected families per cycle, and the crossing method for each generation are listed in Table 2. All recombination and selfing experiments were conducted under WW environments at the maize experiment station in Kiboko, Kenya.

Development of Lines via Pedigree Selection

The top eight C_0 families selected for MARS were also advanced to $F_{5:6}$ through pedigree method under WW environments with visual selection (Fig. 1). For each population, phenotypic selection within and among families was made in Kibos, Kenya, based on per se visual evaluation (germination and good stand establishment, plant type, low ear placement, and well-filled ears) and reaction to naturally occurring major leaf diseases such as gray leaf spot caused by *Cercospora zeae-maydis* Tehon and E.Y. Daniels, leaf blight caused by *Exserohilum turcicum* (Pass.) Leo and Suggs, common rust caused by *Puccinia sorghi* Schw., and maize streak virus caused by maize streak *geminivirus*. The selected $F_{2:3}$ plants were selfed to form $F_{3:4}$ lines and planted at Kiboko, Kenya, at a high plant density (80,000 plants ha^{-1}). Plants with less root and stalk lodging and low ear placement were selfed to form $F_{4:5}$ lines. The same procedure was repeated to form $F_{5:6}$ lines. The best five $F_{5:6}$ lines were selected from each population, top-crossed to a single-cross tester, and included in the genetic gain studies.

Genetic Gain Studies

A total of seven groups of entries from each of the 10 populations (C_0, C_1, C_1S_1, C_1S_2, founder parents, F_1, and pedigree selected lines) plus five commercial checks widely used in SSA (WH504, WH505, CZH0616, DK8053, and Pioneer 3253) were used for the genetic gain studies. For each population, each entry was represented by a balanced bulk of seeds (184 families for C_0, eight families for C_1, 23 families for C_1S_1, and 92 families for C_1S_2) and five lines developed through pedigree selection. Each entry was test crossed with a single tester (CML395/CML444) from a complementary heterotic group. The test crosses from the seven groups of entries, along with the five commercial checks listed above, were evaluated in four WW (Embu, Kakamega, Kiboko, and Kirinyaga) and four WS (Kiboko, Mtwapa, Mbeere-1, and Mbeere-2) environments in Kenya. Experiments were planted in α-lattice design with three replications per environment. At all environments, entries were planted in two-row plots of 5 m long spaced 0.75 m between rows and 0.25 m between hills. Two seeds per hill were planted and thinned to one plant per hill 3 wk after emergence to achieve a final density of 53,333 plants ha^{-1}. Fertilizers were applied at the rate of 60 kg N and 60 kg P_2O_5 ha^{-1} as recommended for the area. Nitrogen was split-applied at planting and 6 wk after emergence. Fields were kept free of weeds by hand weeding. The WS and WW evaluations were conducted as described above for the C_0 populations. Data was collected for GY, plant height (PH), anthesis date (AD), and ASI. Anthesis date was recorded as the number of days from planting to when 50% of the plants had shed pollen, while silking date (SD) was recorded as the number of days from planting to when 50% of the silk emerged. The ASI was calculated as the difference between SD and AD. Plant height was measured as the distance from the base of the plant to the height of the first tassel branch. In WS experiments, all ears were harvested from each plot, shelled, and weighed to determine yield and percentage grain moisture. In the WW experiments, ears harvested from each plot were weighed, and moisture content was determined on a subsample of grain. For both WS and WW environments, GY was estimated by adjusting to 12.5% (125 g kg^{-1}) moisture content.

An analysis of variance for GY, AD, ASI, and PH within WS and WW environments was performed for each location and combined across environments for each water management using the PROC MIXED procedure of SAS 9.3 (SAS Institute, 2009) considering environments and incomplete blocks as random effects and recovering interblock information. Entries were considered fixed effects. Response to selection was determined by regressing mean values to the breeding cycle means (C_0, C_1, C_1S_1, and C_1S_2) for each population and across all populations combined over WS and WW environments. Since the groups of entries (bulks of the C_0, C_1, C_1S_1, C_1S_2, founder parents, F_1, commercial checks, and five $F_{5:6}$ lines developed through conventional pedigree selection) had different sample sizes, the least significant difference (LSD) was calculated using the harmonic mean for each group.

RESULTS AND DISCUSSION

Genetic Gains across Ten Biparental Populations in Water-Stressed Environments

Table 3 summarizes GY, AD, ASI, and PH for the eight groups of entries evaluated under WS and WW environments. The mean GY across the 10 populations in the WS experiments ranged from 1.94 to 2.68 Mg ha^{-1}, with an overall mean of 2.47 Mg ha^{-1}. The GY of C_1S_2 (2.68 Mg ha^{-1}) was significantly ($P < 0.05$) greater than that of C_0 (2.51 Mg ha^{-1}), founder parents (2.48 Mg ha^{-1}), and commercial checks (1.94 Mg ha^{-1}). Hybrids derived from C_1S_2 produced 3.8 and 5.9% greater GY than hybrids from C_1, and C_1S_1, respectively, but this was not statistically significant.

Hybrids derived using lines from pedigree selection produced 2.53 Mg ha^{-1}, which is 5.6% lower than those hybrids from the C_1S_2. Although we used GY as the target trait for the genetic gain studies, we also evaluated AD, ASI, and PH to understand if significant differences occurred for these traits during selection. Hybrids derived from C_1S_2 lines did not significantly differ in AD (maturity) and ASI from other groups of entries, but they were significantly ($P < 0.05$) taller (5.3–11.2 cm) than those hybrids derived from C_0, founder parents, pedigree lines, and commercial checks (Table 3). In another study (Beyene

Table 3. Means of eight groups of entries (C_0, C_1, C_1S_1, C_1S_2, F_1, founder parents, pedigree, and commercial checks) across 10 marker-assisted recurrent selection populations evaluated under four water-stressed (WS) and four well-watered (WW) environments in sub-Saharan Africa for grain yield (GY), anthesis date (AD), anthesis silking interval (ASI), and plant height (PH). For each trait, the highest values for each environment are in bold. Least significant differences at the 0.05 probability level ($LSD_{0.05}$).

	Water-stressed				Well-watered			
Group	GY	AD	PH	ASI	GY	AD	PH	ASI
	Mg ha^{-1}	d	cm		Mg ha^{-1}	d	cm	
C1S2	**2.68**	63.25	**186.60**	1.03	**7.15**	67.27	240.78	1.13
C1S1	2.52	64.02	184.75	0.81	7.02	67.70	242.62	0.83
C_1	2.58	63.93	184.54	0.93	7.05	67.11	**245.23**	0.88
C_0	2.51	62.73	179.23	1.30	6.52	66.72	235.00	1.39
F_1	2.54	62.83	177.66	1.25	6.63	66.43	233.58	1.43
Parents	2.47	62.98	175.38	1.30	6.73	66.32	234.14	0.95
Pedigree	2.53	63.60	181.32	0.97	6.90	**67.55**	238.40	1.18
Checks	1.94	**64.23**	179.30	**1.93**	5.99	67.28	244.62	**2.13**
$LSD_{0.05}$	0.17	0.83	5.03	0.45	0.33	0.83	4.89	0.43

et al., 2015), we have reported a high potential of GS in increasing GY under WS environments in SSA without significantly affecting AD and PH in most populations. In that study, hybrids developed from C_3 of GS produced 8.4 and 19.5% greater GY than hybrids developed from C_0 and founder parents, respectively. Our results from the two studies showed slight advantage for GS over MARS in improving GY under WS environments.

Using the test crosses to founder parental lines as the baseline, C_1S_2–derived hybrids showed a total gain of 0.205 Mg ha^{-1}. As shown in Fig. 1, a total of nine seasons over 4 yr were needed from development of F_1s to harvesting of C_1S_2, with two seasons per year needed to go from F_1 to C_0 test cross evaluation and three seasons per year needed for C_1, C_1S_1, and C_1S_2. The gain per year with the MARS scheme was, therefore, 51 kg ha^{-1} yr^{-1} (Table 4), which is 28% lower than the 70.5 kg ha^{-1} yr^{-1} reported in our previous study through GS (Beyene et al., 2015). Massman et al. (2013) compared the response of GS and MARS on stover and GY in a biparental maize population derived from a cross between B73 and Mo17. That study involved genotyping 233 RILs with 284 markers, evaluating the test crosses under WW environments, and advancing the population using GS and MARS; the authors reported 14 to 50% greater response to GS than MARS.

Preliminary estimates of GY gains from conventional selection in SSA revealed 18 kg ha^{-1} yr^{-1} under WS (Edmeades, 2013). A recent study using 67 hybrids developed and released at CIMMYT between 2000 and 2011 showed genetic gains of 32 kg ha^{-1} yr^{-1} for GY under WS (B. Masuka, personal communication, 2015). Therefore, the average gain for GY observed under drought in our study using MARS was 1.6 to 2.8 times greater than what has been reported from conventional phenotypic selection in SSA but slightly lower than the gain using GS reported by Beyene et al. (2015).

Table 4. Estimated genetic gain of C_1S_2–derived hybrids across 10 marker-assisted recurrent selection populations using their founder parents as baseline data.

Entry type	Water-stressed	Well-watered
C_1S_2 (Mg ha^{-1})	2.68	7.15
Parents (Mg ha^{-1})	2.47	6.73
Gain (Mg ha^{-1})	0.205	0.420
No. of years	4.00	4.00
Gain per year (Mg ha^{-1})	0.051	0.105

Genetic Gains across Ten Populations in Well-Watered Environments

As shown in Table 3, mean GY across populations and entry types in WW environments varied from 5.99 to 7.15 Mg ha^{-1}, with an overall mean of 6.75 Mg ha^{-1}. The WS environments have therefore reduced mean GY by 63.4% (range: 61.5–67.5% per population) compared with the WW environments, which is close to the 70% yield reduction that is typically targeted by CIMMYT breeders in SSA (Bänziger et al., 2000). In the WW environments, the GY of C_1S_2 (7.15 Mg ha^{-1}) was significantly ($P < 0.05$) greater than that of C_0 (6.52 Mg ha^{-1}), founder parents (6.73 Mg ha^{-1}), and commercial checks (5.99 Mg ha^{-1}). Hybrids derived from C_1S_2 produced 3.4, 1.8, and 1.3% greater GY than those hybrids derived from pedigree selected lines, C_1S_1, and C_1, respectively, but it was not statistically significant. Hybrids derived from C_1S_2 were significantly ($P < 0.05$) taller than the hybrids derived from C_0 and founder parents, but there was no significant difference in maturity with all groups of entries, which shows that the increase in GY for C_1S_2–derived hybrids over the others was not at the expense of an increase in the crop's time to maturity.

The average gain in GY in WW environments was 0.420 Mg ha^{-1}, which is equivalent to 105 kg ha^{-1} yr^{-1} (Table 4). The gain in GY under WW environments was therefore twofold greater than the gain in GY of the same hybrids evaluated in WS environments. This is due to the lower genetic variance and heritability of GY under WS environments. These findings were in agreement with other studies that reported reduced genetic variance and heritability of GY with increased moisture stress (Bolaños and Edmeades 1996; Rosielle and Hamblin 1981; Lafitte and Bänziger 1997; Beyene et al., 2013). A review of the genetic gain studies from conventional pedigree selection conducted in tropical maize germplasm reported highly variable results, which varied from 39 to 80 kg ha^{-1} yr^{-1} (Edmeades, 2013) to 109 kg ha^{-1} yr^{-1} (B. Masuka, personal communication, 2015). In the United States, GY gains during the past 70 yr varied from 65 to 75 kg ha^{-1}

Table 5. Means of eight groups of entries (C_0, C_1, C_1S_1, C_1S_2, F_1, founder parents, pedigree, and commercial checks) for each of the 10 marker-assisted recurrent selection populations evaluated under four water-stressed and four well-watered environments in sub-Saharan Africa for grain yield (GY), anthesis date (AD), and plant height (PH). The highest values for each trait and environment are in bold. Least significant differences at the 0.05 probability level ($LSD_{0.05}$).

		Water-stressed			Well-watered		
Population	Group	GY	AD	PH	GY	AD	PH
		Mg ha^{-1}	d	cm	Mg ha^{-1}	d	cm
6x1008	C_1S_2	**2.97**	62.83	191.17	**7.32**	66.25	**250.50**
	C1S1	2.78	63.00	189.08	7.14	66.08	243.38
	C_1	2.58	**63.33**	183.67	7.13	66.42	243.46
	C_0	2.36	**63.33**	179.90	6.23	**67.08**	232.00
	F_1	2.58	62.00	182.55	6.32	66.50	234.63
	Parents	2.76	62.17	179.30	7.16	65.13	239.75
	Pedigree	2.74	62.97	**191.42**	6.83	66.43	249.24
	$LSD_{0.05}$	0.35	0.70	13.53	0.93	0.78	9.74
6x1015	C_1S_2	**2.91**	62.83	**193.95**	**7.49**	66.33	253.33
	C_1S_1	2.47	**64.17**	187.03	6.69	66.08	248.96
	C_1	2.70	63.33	189.85	7.42	**66.75**	**260.63**
	C_0	2.74	61.33	177.75	6.30	65.67	246.04
	F_1	2.81	63.00	183.12	6.29	66.00	240.46
	Parents	2.81	62.25	178.13	6.50	65.58	237.19
	Pedigree	2.76	62.40	185.11	6.73	66.73	238.68
	$LSD_{0.05}$	0.40	1.09	12.37	0.72	0.88	8.78
6x1016	C_1S_2	**2.97**	63.00	200.13	7.29	68.00	251.54
	C_1S_1	2.34	**66.00**	197.56	6.71	66.15	251.67
	C_1	2.78	62.50	191.43	8.00	66.17	**267.63**
	C_0	**2.97**	62.33	**203.25**	7.37	67.42	263.21
	F_1	2.41	63.33	190.82	7.08	65.92	251.42
	Parents	2.61	63.58	191.54	**8.16**	67.33	251.27
	Pedigree	2.65	64.47	202.13	7.74	**68.22**	258.69
	$LSD_{0.05}$	0.52	2.09	7.33	0.75	1.55	11.34
6x1017	C_1S_2	2.87	61.83	197.07	**8.65**	66.83	252.33
	C_1S_1	2.95	63.50	198.22	7.81	66.25	255.63
	C_1	**3.19**	**68.33**	**201.60**	7.29	66.67	**256.42**
	C_0	2.83	63.50	188.28	7.71	**67.17**	252.58
	F_1	2.65	62.33	182.90	7.27	65.50	245.21
	Parents	2.74	62.50	186.35	7.09	66.33	239.48
	Pedigree	2.85	63.00	190.12	7.45	66.97	249.11
	$LSD_{0.05}$	0.50	2.66	10.83	1.06	1.49	8.70
6x1018	C_1S_2	2.69	64.50	193.43	7.68	**68.92**	252.21
	C_1S_1	2.30	**64.83**	**194.00**	7.62	68.42	254.25
	C_1	2.53	63.83	193.00	**7.86**	67.33	**267.71**
	C_0	**2.89**	63.33	190.60	7.50	67.50	232.42
	F_1	2.64	63.17	184.42	6.85	67.75	241.25
	Parents	2.42	63.75	181.85	6.85	68.00	237.19
	Pedigree	2.64	63.90	192.02	7.47	68.00	252.12
	$LSD_{0.05}$	0.52	1.79	13.93	0.44	1.46	8.37

(cont'd.)

yr^{-1} (Duvick, 2005). Eathington et al. (2007) reported that the genetic gain for GY using MARS was double that of the pedigree selection methods in the temperate maize breeding populations. Therefore, the 105 kg ha^{-1} yr^{-1} gain observed under WW environments in our study using MARS was 24 to 33% greater than the results reported by Duvick (2005) and Edmeades (2013) but lower than what has been report by Eathington et al. (2007).

Genetic Gains for Each Biparental Population in Drought Environments

Table 5 shows the GY of each of the 10 populations evaluated under WS and WW environments. Our results in WS environments demonstrated fairly consistent genetic gain rates, with eight populations showing 1.4 to 25.8% (mean = 10.5%) greater GY for hybrids derived from C_1S_2 than C_0, one population (6x1016) showing zero gain and another population (6x1018) showing a 7.2% reduction in

Table 5. Continued.

Population	Group	Water-stressed			Well-watered		
		GY	AD	PH	GY	AD	PH
		Mg ha^{-1}	d	cm	Mg ha^{-1}	d	cm
6x1019	C_1S_2	**2.47**	60.50	175.80	5.32	64.75	**221.29**
	C_1S_1	2.25	**61.17**	167.42	5.25	64.17	220.25
	C_1	2.07	60.67	**177.85**	**5.85**	**64.92**	217.67
	C_0	2.36	59.50	163.05	5.31	64.25	202.88
	F_1	2.38	59.17	166.45	5.43	61.75	215.17
	Parents	2.12	59.92	164.39	5.07	63.21	210.46
	Pedigree	2.21	60.90	168.54	5.46	64.52	215.78
	$LSD_{0.05}$	0.39	2.78	12.11	0.94	1.80	11.28
6x1020	C_1S_2	2.64	61.50	172.83	6.49	65.33	222.67
	C_1S_1	2.39	62.33	173.30	6.63	**73.50**	226.83
	C_1	**2.80**	**62.50**	168.12	6.62	65.25	230.38
	C_0	2.50	60.50	174.87	5.85	64.67	225.42
	F_1	2.40	62.33	171.98	6.44	65.58	220.67
	Parents	2.17	62.25	165.53	6.35	64.33	231.06
	Pedigree	2.44	62.90	**175.82**	**6.72**	66.07	**234.93**
	$LSD_{0.05}$	0.35	1.40	11.92	1.03	0.63	9.68
6x1021	C_1S_2	2.37	**63.50**	168.18	6.24	65.50	217.92
	C_1S_1	**3.01**	62.33	173.28	**6.54**	65.75	**227.63**
	C_1	2.62	62.67	**174.12**	5.85	65.83	223.13
	C_0	2.15	62.17	163.43	5.56	64.75	210.25
	F_1	2.60	62.50	162.00	5.82	65.08	209.21
	Parents	2.35	61.75	158.08	5.53	64.42	214.21
	Pedigree	2.47	63.07	157.39	6.03	**66.15**	211.20
	$LSD_{0.05}$	0.55	1.43	15.40	0.75	1.05	6.72
6x1023	C_1S_2	**2.53**	65.50	**188.63**	7.45	**70.42**	236.54
	C_1S_1	2.38	66.17	188.05	**8.48**	68.50	**247.63**
	C_1	2.36	65.67	184.92	7.74	69.33	242.50
	C_0	2.27	**66.50**	179.38	6.75	69.08	239.38
	F_1	2.89	64.67	182.87	7.69	70.17	241.58
	Parents	2.57	66.00	174.47	7.872	69.46	245.73
	Pedigree	2.32	66.13	173.25	7.432	70.88	235.13
	$LSD_{0.05}$	0.64	1.44	5.19	1.354	1.49	9.17
6x1028	C_1S_2	**2.37**	66.50	**184.78**	**7.53**	70.42	**249.46**
	C_1S_1	2.33	**67.00**	181.70	7.34	72.25	249.21
	C_1	2.13	66.50	180.88	6.72	**72.42**	242.75
	C_0	1.99	64.83	171.78	6.65	69.58	245.83
	F_1	2.02	65.83	169.50	7.15	70.00	236.25
	Parents	2.19	65.67	174.19	6.66	69.38	235.05
	Pedigree	2.21	66.30	177.39	7.19	71.48	239.10
	$LSD_{0.05}$	0.46	1.33	15.57	0.63	1.29	8.67

GY. All C_1S_2 hybrids had <1.5 d difference in flowering time compared with hybrids derived from C_0. A similar trend in genetic gain in GY was also observed when the performance of C_1S_2 hybrids was compared with that of hybrids developed from the founder parents and the pedigree method. Nine populations showed an increase of 0.8 to 21.5% (mean = 9.8%) and 1.0 to 12.1% (mean = 7.3%) over founder parents and pedigree selection, respectively. Only populations 6x1023 and 6x1021 showed reductions of 1.3 and 3.9% for C_1S_2 hybrids compared with the founder parents and pedigree selection, respectively. Plant height of C_1S_2–derived hybrids was not significantly different from that of hybrids developed from C_0, pedigree and founder parents (Table 5).

Genetic Gains for Each Biparental Population in Well-Watered Environments

C_1S_2 hybrids from eight populations showed from 2.4 to 18.9% (mean = 12.2%) greater GY than those derived from C_0 (Table 5). Population 6x1016 showed a GY reduction of about 1% for C_1S_2–derived hybrids compared with C_0, while population 6x1019 showed nearly zero gain in GY. C_1S_2 hybrids derived from all populations, except two, showed an increase of 2.1 to 22.1% (mean = 10.6%) as compared with

hybrids derived from the founder parents; only populations 6x1016 and 6x1023 showed GY reductions of 10.7 and 5.4%, respectively (Table 5). Compared with hybrids derived using the pedigree method, C_1S_2 hybrids from seven populations gave 2.9 to 16.8% (mean = 7.7%) greater GY. For all populations, the difference in AD was <1.5 d.

Overall results of the individual MARS populations revealed differential response, with the majority of the populations showing greater GY for C_1S_2–derived hybrids than the other group of entries, but few populations showed better GY either at C_1S_1 or C_1 (Table 5). Such differential response to selection was observed in both WW and WS environments. We have no firm explanation for this puzzling phenomenon but hypothesize that it may be related to the fact that the C_1 individuals selected were highly heterozygous relative to both the $F_{2:3}$ (C_0) and the C_1S_1 families. Nevertheless, our study involved a large dataset and provides highly relevant information to maize breeders planning to use MARS and GS for developing stress tolerance in maize.

In addition to evaluating genetic gains from different cycles of selection, a total of 840 C_3S_5 lines and 352 doubled-haploid lines have been developed from each cycle for all 10 biparental populations and tested in multilocation trials. Several hybrids were derived using lines developed through MARS and pedigree methods. The best hybrids from each population are currently under national performance trials and we are expecting release of some hybrids soon for commercialization in SSA.

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