

Use of Genomic Estimated Breeding Values Results in Rapid Genetic Gains for Drought Tolerance in Maize

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Abstract

More than 80% of the 19 million ha of maize (*Zea mays* L.) in tropical Asia is rainfed and prone to drought. The breeding methods for improving drought tolerance (DT), including genomic selection (GS), are geared to increase the frequency of favorable alleles. Two biparental populations (CIMMYT-Asia Population 1 [CAP1] and CAP2) were generated by crossing elite Asian-adapted yellow inbreds (CML470 and VL1012767) with an African white drought-tolerant line, CML444. Marker effects of polymorphic single-nucleotide polymorphisms (SNPs) were determined from testcross (TC) performance of $F_{2:3}$ families under drought and optimal conditions. Cycle 1 (C1) was formed by recombining the top 10% of the $F_{2:3}$ families based on TC data. Subsequently, (i) C2[PerSe_PS] was derived by recombining those C1 plants that exhibited superior per se phenotypes (phenotype-only selection), and (ii) C2[TC-GS] was derived by recombining a second set of C1 plants with high genomic estimated breeding values (GEBVs) derived from TC phenotypes of $F_{2:3}$ families (marker-only selection). All the generations and their top crosses to testers were evaluated under drought and optimal conditions. Per se grain yields (GYs) of C2[PerSe_PS] and that of C2[TC-GS] were 23 to 39 and 31 to 53% better, respectively, than that of the corresponding F_2 population. The C2[TC-GS] populations showed superiority of 10 to 20% over C2[PerSe-PS] of respective populations. Top crosses of C2[TC-GS] showed 4 to 43% superiority of GY over that of C2[PerSe_PS] of respective populations. Thus, GEBV-enabled selection of superior phenotypes (without the target stress) resulted in rapid genetic gains for DT.

Core Ideas

- Use of GS for improvement to drought tolerance in tropical maize in Asia is relatively new.
- Positive selection response can be obtained with the use of markers for grain yield under drought.
- Statistical model used for determining marker effects works in practice and thus stands validated.
- GEBV-based selection of superior phenotypes (without target stress) leads to rapid genetic gains.

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Abbreviations: ASI, anthesis silking interval; BLUP, best linear unbiased predictor; C1, Cycle 1; Cycle 2, C2; CAP, CIMMYT-Asia population; CML, CIMMYT maize line; DT, drought tolerance; GEBV, genomic estimated breeding value; GS, genomic selection; GY, grain yield; MARS, marker-assisted recurrent selection; OPV, open-pollinated variety; SNP, single-nucleotide polymorphism; TC, testcross.

MAIZE AREA in Asia (and especially in India) expanded steadily at $>2.2\%$ yr⁻¹ from 2001 to 2007 (Directorate of Economics and Statistics, <http://eands.dacnet.nic.in>). One of the drivers for this expansion was the increased demand for maize feed (a result of a shift toward meat-based diets—an indicator of higher per capita income made possible by changes in governmental policy that encouraged open market entrepreneurship). Maize in China covers 35 million ha, while in southern Asia (India, Bangladesh, Sri Lanka, Pakistan, Nepal, and Bhutan) and southeastern Asia (Philippines, East Timor, Indonesia, Cambodia, Laos, Vietnam, Myanmar, and Thailand) it covers 20 million ha, of which 8 million ha is in India (faostat.fao.org). Approximately 70% of the maize in Asia is used as feed, and 70% of this feed is used for poultry. Yellow is the preferred maize grain color for the feed industry.

The importance of drought in Asia and its effect on maize productivity began to be recognized in the early 1990s, as $>80\%$ of the maize in Asia is rainfed and hence prone to climatic variability that leads to deficit moisture (or agricultural drought) at critical crop stages. In general, across the globe, and particularly in Asia, of all the stresses experienced by a maize crop during the crop cycle, drought reduces yield by $>20\%$ (Edmeades et al., 2003). At CIMMYT (Mexico), breeding for DT started in the 1980s. Drought-tolerant germplasm from Mexico flowed into Africa in the mid-1990s, and the success of this drought breeding work was reported by Bänziger et al. (2006). Inbred lines tolerant to drought were released as CIMMYT maize lines (CMLs) and open-pollinated varieties (OPVs) and hybrids reached farmers' fields by the mid-2000s. Highly elite CIMMYT inbreds—whose resultant hybrids yielded 1 t ha⁻¹ more than elite private-sector germplasm under drought—was made available to researchers (Bänziger and Araus, 2007). However, in Asia, the public maize breeding programs had, up to the mid-2000s, focused on developing maize germplasm for high yield potential and disease resistance. Specialty maize varieties, like sweet corn and baby corn, were of commercial importance in specific pockets in Asia (Thailand) with market needs served by both public and private breeding programs. CIMMYT's tropical Asian maize germplasm was originally bred in Thailand with emphasis on high yield potential, lodging tolerance, disease resistance (*Turcicum* leaf blight and downy mildew), and nutritional quality (quality protein maize). Breeding emphasis expanded to developing resistance to gray leaf spot and banded leaf and sheath blight. Very little or no emphasis had been placed on developing tolerance to abiotic stresses like drought and heat. Hence, through this effort, the requirement for DT maize in Asia had to

be met with introgressions of DT from the largely white-kernelled sources from Mexico and Africa into elite yellow Asian backgrounds.

Drought tolerance is a highly polygenic trait with significant additive effects of many chromosomal regions. Thus, effective introgression of DT involves the transfer of most of these chromosomal regions into the target germplasm. Marker-assisted recurrent selection (MARS)—a technology designed to increase the frequency of additively inherited favorable alleles in elite breeding populations either by selecting among recombinants of F₂ or back cross progenies—was known to be especially effective for polygenic traits (Bernardo, 2008). In 2007, the African programs began to enhance DT levels in their best lines using MARS (Beyene et al., 2016), a technology that was being increasingly and successfully used in the private industry (Eathington et al., 2007). Genetic gains for complex traits, such as GY, grain moisture, or standability, were being doubled through these approaches.

Thus, it was expected that similar approaches could assist in accelerated introgression of DT into Asian germplasm, provided the most elite germplasm and state-of-the-art (and continuously evolving) technologies would be used. It was known (Ribaut and Ragot, 2007) that knowledge and careful selection of parental germplasm, accuracy of phenotyping, and effective and responsive use of genotypic, phenotypic, and genotype \times environment interaction information would be key to success for maximizing breeding progress. Beyene et al. (2016) reported a gain of 0.051 t ha⁻¹ using MARS across 10 populations in sub-Saharan Africa. Also, Beyene et al. (2015) reported average gains per selection cycle of ~ 0.089 t ha⁻¹ in eight biparental population with two cycles of GS under drought and demonstrated that GS was more effective than pedigree-based conventional phenotypic selection for increasing genetic gains in GY under drought stress in tropical maize. To our knowledge, there are no reports describing the use of GS for improvement of DT in tropical maize in Asia. Therefore, the objective of this study was to report on gains made through GS and to compare breeders' practices of developing improved source populations through S₁ testcrosses and subsequent per se selections with that of GS.

Materials and Methods

Formation of Genomic Selection Populations

All pollinations (selfing and crossing) were made at ICRISAT, Hyderabad. From a large number of putative pedigree single (biparental) crosses, two crosses were selected for improvement through GS, in which genome-wide markers were used to make marker-only selections. The selection of these crosses was based on the results of a Design II between 25 yellow Asian inbred lines with seven African white drought-tolerant donor lines (results not included). Besides India, these trials were evaluated by partners in China, Thailand, Vietnam, Philippines,

and Indonesia. Based on this evaluation, CML444 turned out to be the best drought-tolerant donor and was used in two of three crosses. Elite lines from the CIMMYT-Asia program (CAP), CML470 and VL1012767, were chosen for improvement. A summary of these populations is in Table 1. Upon selfing, F_2 populations of these crosses were obtained and were subject to further processes as per the GS flowchart (Fig. 1).

The $F_{2,3}$ families from each population were crossed to tester CML474. All the above crosses tended to show the B-type heterotic pattern (flint-type kernels); hence the A-type tester (dent-type kernels), CML474, was used for generating the testcrosses.

Phenotyping

Testcrosses were divided into several trials based on seed availability and were phenotyped in one to three drought and one to three well-watered environments (Table 2). The CAP1 and CAP2 per se trials were evaluated at ICRI-SAT, Sabour, Ludhiana and Jalna. The CAP1 testcrosses were evaluated at ICRISAT, Sabour, and Ludhiana. The CAP2 testcrosses were evaluated at ICRISAT, Sabour, Belgaum, and Davanagere. The experiments were planted as α -lattice design with two to three replications per location. The managed drought-stress trials were conducted during the dry season. A saturating irrigation was given when the crop reached 550 growing degree days. Drought stress at flowering to mid-grain fill was imposed on the crop by withdrawing subsequent irrigations. Soil moisture content (v/v), up to a soil depth of 1 m, was monitored weekly with a soil moisture probe (Delta T Devices Profile Probe PR2/6) by placing one access tube for approximately every 60 plots ($\sim 225 \text{ m}^2$). Irrigation was resumed just before the soil reached permanent wilting point between the 30- and 40-cm soil depths. Entries were planted in one- to two-row plots of 4 m at a spacing of 0.75 m between rows and 0.2 m between plants. Plots were oversown and were later thinned to ensure a plant population of 63,636 plants ha^{-1} . Fertilizer application was based on the local fertilization practice of each location, which was aligned with the recommendations given by the Department of Agriculture of each respective state. Plots were kept weed free by manual weeding and interculture. All normal agronomic traits were recorded for the trials including dates of anthesis and silking, plant height, and ear height.

Phenotypic data for each site were analyzed using the residual maximum likelihood procedure in Fieldbook (Vivek et al., 2007). Grain yield was estimated by adjusting the moisture to 12.5%. Best linear unbiased predictors (BLUPs) were calculated for each entry to assess their performances across sites. Best linear unbiased predictors were calculated for plant-stand adjusted GY for both drought sites and optimal sites and for anthesis silking interval (ASI) under drought.

Genotyping

Parents of promising CAPs were surveyed for polymorphism with 1214 SNP markers using KASPar assay at

Table 1. Summary of CIMMYT-Asia populations (CAPs) used in this study.

Population	Parent1	Parent2	$F_{2,3}$ population size	Polymorphic single-nucleotide polymorphisms
CAP1	CML470	CML444	276	342
CAP2	VL1012767	CML444	178	377

LGC Genomics, UK. The $F_{2,3}$ progenies of CAP1 and CAP2 were also genotyped at LGC Genomics with SNPs polymorphic for respective populations. The number of progenies and SNPs genotyped in each of CAP populations are listed in Table 1.

Marker-assisted recurrent selection projects from previous studies at CIMMYT using African populations had not shown major or significant quantitative trait loci for yield under drought (Tuberosa et al., 2007; Araus et al., 2008), hence, the approach of GS, where all marker effects are incorporated in a GEBV (Meuwissen et al., 2001), was explored in this project. This method is known to avoid selection bias associated with significance tests and captures more genetic variability for highly polygenic traits (Bernardo, 2008).

Formation of Cycle 1

A selection index was calculated on BLUPs from testcross data and weighted as follows: 35% GY under drought, 25% ASI under drought, and 40% GY under optimal conditions. For the formation of C1, the top 10% of the F_3 families were selected based on the above selection index (i.e., phenotypic data only) and then recombined. Marker effects for all polymorphic markers in a population were calculated by correlating the testcross phenotypic performance with genotypic data of respective $F_{2,3}$ families using R script (BLR package of R software).

Formation of Cycle 2

Three hundred and fifty seeds of C1 were planted. Each plant was individually tagged and leaves were sampled. Dried leaves were sent to LGC Genomics with a list of SNPs to be genotyped for each population. The SNPs used for genotyping the C1 plants were the ones whose marker effects were determined in the first round of genotyping. Based on genotyping data, GEBVs were calculated (Cossa et al., 2010) for each of the sampled plants. A larger GEBV indicated a favorable plant. Plants with top GEBVs (24–30 plants or $\sim 10\%$ selection intensity) were identified and were recombined. Such populations were termed C2[TC-GS] (for C2 generated by GS using marker effects generated from testcross data).

On a second set of C1 plants grown under optimal conditions, visually appealing plants were used for recombination to generate C2[PerSe-PS] (for C2 generated by per se phenotype). Normally, while selecting for DT, the evaluation and the recombination units are kept separately; the superior families identified in the evaluation units under drought are recombined under

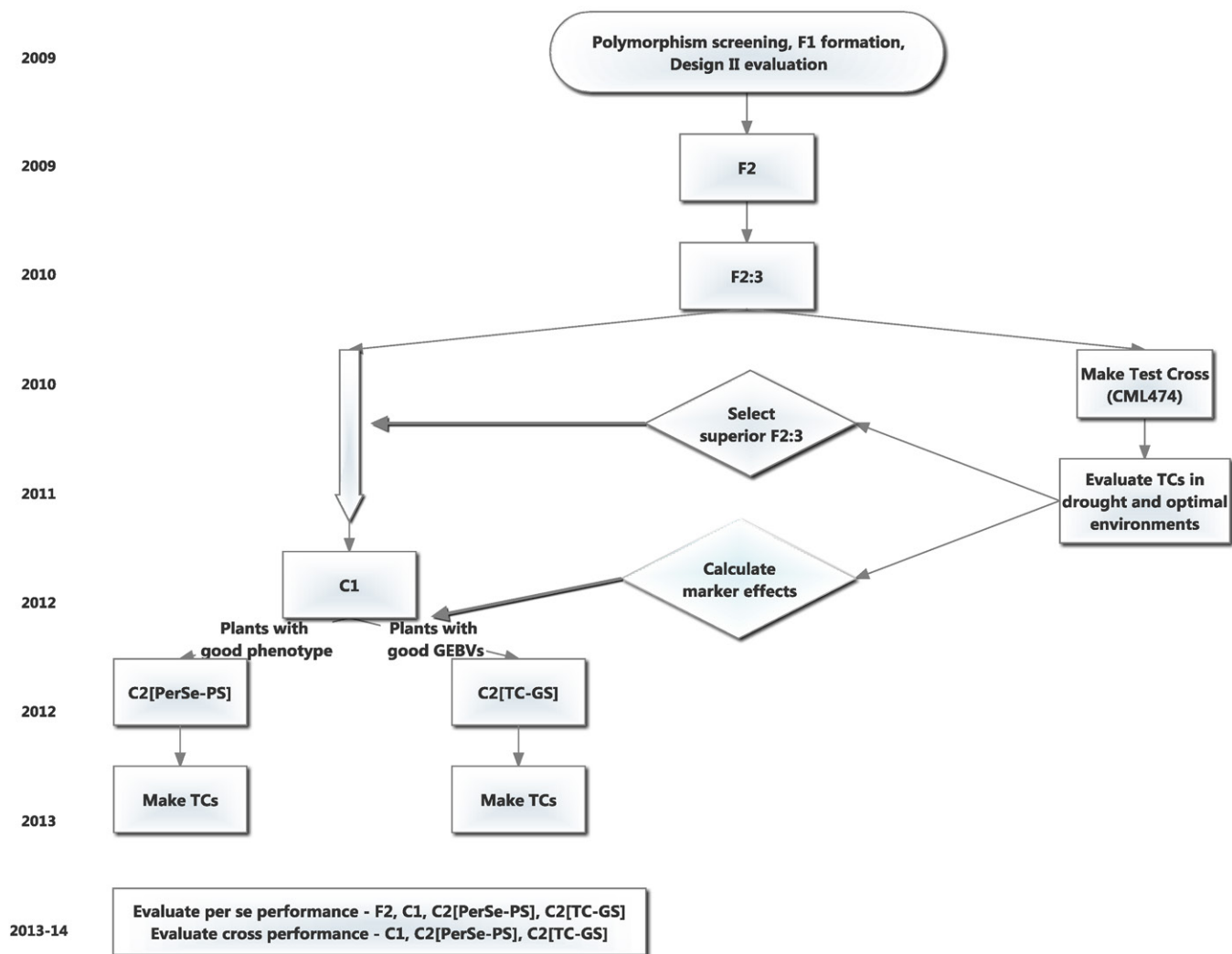


Fig. 1. Work flow summary of the genomic selection procedures used in the development of various improved cycles of selection.

optimal conditions (the recombination units). In this case, each C1 plant was unique (generated by crossing of the segregating F_3 plants), with only one chance for selection and recombination. Given the work flow of the technology, time, and resources, only a limited quantity of seed of C1 could be generated. However, more importantly, generation of seed under drought stress is unviable given the severity of the stress that is required

Table 2. Details of locations in India and management used for evaluation.

Location	Latitude	Longitude	Trials	Soil Type
ICRISAT-Hyderabad, Telangana	78°16' E	17°30' N	drought, optimal	shallow black with more clay
Sabour, Bihar	87°5' E	25°23' N	optimal	sandy loam with low carbon
Ludhiana, Punjab	75°79' E	130°97' N	optimal	alluvial sandy loam
Jalna, Maharashtra	75°88' E	19°84' N	drought	mix of red and black with low humus
Belgaum, Karnataka	74°50' E	15°85' N	optimal	medium black
Davanagere, Karnataka	75°80' E	14°51' N	drought	red silt loam

for meaningful selection. Hence, breeders resort to making a second recombination under optimal conditions and, in the process, also select for as many traits that are visually appealing.

For selection of visually appealing plants, the traits considered were as follows: standability (no lodging), vigor, general plant aspect including ear position, and diseases. Further, visually appealing cobs were selected based on ear rots, texture, color, general ear aspect, grain fill, and cob size.

Genetic Gains Study

At least 200 plants of each cycle of selection were planted. Bulk pollen of these plants represented the population and was used for pollinating on to tester CML474 (as female), the original tester used with the $F_{2:3}$ families to determine marker effects.

Trials containing these crosses and the various cycles of selections were separately evaluated for cross and per se performance, respectively, under 1–3 optimal and 1–3 drought environments (Table 2).

Results

Per Se Performance of Cycles of Selection

Grain Yield

Based on average GY across the drought locations and two populations, per se performance of C2[PerSe-PS] (representing phenotypic selection) ranged from 32 to 39%, while that of C2[TC-GS] (representing genomic selection) ranged from 53 to 59% compared with the F_2 of the respective population. The per se performance of C2[TC-GS] was 10 to 20% better than C2[PerSe-PS] (Table 3). Genetic gain per cycle for phenotypic selection ranged from 0.27 to 0.31 t ha⁻¹, while that of GS ranged from 0.42 to 0.5 t ha⁻¹. Genetic gain per year for phenotypic selection ranged from 0.067 to 0.076 t ha⁻¹ yr⁻¹, while that of GS ranged from 0.104 to 0.124 t ha⁻¹ yr⁻¹ (Table 3). The C2[PerSe-PS] population was formed based on plant performance under optimal conditions, whereas C2[TC-GS] was formed—using marker effects—which were derived from testcross performance under drought and optimal conditions.

Genetic gain per cycle phenotypic selection is known to be better than GS (Bernardo and Yu, 2007; Bernardo, 2008) especially for per se performance. But in our case, we are comparing two populations: one was constituted based on individual plant performance under optimal conditions and the other was based on marker effects derived from testcrosses under drought and optimal conditions. Since both these cycles are being compared under drought, the genetic gain per cycle appears to be more for GS than for phenotypic selection.

Based on an average GY across optimal locations across the two populations, per se performance of

C2[PerSe-PS] (phenotypic selection) ranged from 23 to 27%, while that of C2[TC-GS] (genomic selection) ranged from 31 to 38% compared with the F_2 . Per se performance of C2[TC-GS] was 2 to 12% better than C2[PerSe-PS] (Table 3). The C2[TC-GS] population was formed using marker effects of a selection index that included GY under drought and optimal conditions. Genetic gain per cycle for phenotypic selection ranged from 0.34 to 0.48 t ha⁻¹, while that of GS ranged from 0.53 to 0.55 t ha⁻¹. Genetic gain per year for phenotypic selection ranged from 0.084 to 0.123 t ha⁻¹ yr⁻¹, while that of GS ranged from 0.13 to 0.14 t ha⁻¹ yr⁻¹. Beyene et al. (2015) reported a genetic gain of 0.094 t ha⁻¹ cycle⁻¹ and 0.070 t ha⁻¹ yr⁻¹ for GS under drought. Recurrent selection in several diverse tropical maize populations for tolerance to drought at flowering over two to 10 cycles increased GY under stress by ~0.1 t ha⁻¹ yr⁻¹ and reduced ASI by 0.6 d yr⁻¹ (Edmeades et al., 2000).

Anthesis Silking Interval

Under drought and across locations and populations, genetic gain per cycle for phenotypic selection (C2[PerSe-PS]) of ASI showed a range between -0.05 and 0.5 d, while that of GS (C2[TC-GS]) ranged from -0.33 to 0.25 d. Genetic gain per year for phenotypic selection for ASI ranged from -0.01 to 0.13 d yr⁻¹, while that of GS ranged from -0.08 to 0.06 d yr⁻¹. Under optimal conditions, the ASI for phenotypic and GS was in the optimal range (<2 d), and hence, the comparison of any gain was of less practical significance than ASI reduction under drought. Bolaños and Edmeades (1996) associated selection gains under drought to a reduced ASI. In this study, marginal improvements to ASI were seen. During the development

Table 3. Per se performance and per se genetic gains of cycles of selection.

Generation	Drought						Optimal					
	CAP1†			CAP2			CAP1			CAP2		
	GY‡	Gain over F2 %	ASI§	GY	Gain over F2 %	ASI	GY	Gain over F2 %	ASI	GY	Gain over F2 %	ASI
F_1	3.96		2.5	3.70		3.4	5.68		1.9	4.98		0.3
F_2	1.68		2.6	1.58		1.9	2.87		1.0	3.55		0.0
Cycle 1 (C1)	2.20	31	3.3	2.33	48	2.5	3.85	34	-0.3	3.93	11	-0.8
Cycle 2 (C2) [Per_Se-PS]	2.21	32	2.5	2.19	39	2.9	3.54	23	0.9	4.50	27	0.2
C2 [TC-GS]	2.67	59	1.9	2.41	53	2.4	3.96	38	0.6	4.64	31	0.0
Genetic gain per cycle, phenotypic selection (PS)	0.27		-0.05	0.31		0.50	0.34		-0.05	0.48		0.10
Genetic gain per cycle, genomic selection (GS)	0.50		-0.33	0.42		0.25	0.55		-0.20	0.53		0.00
Gain per year, PS	0.067		-0.012	0.076		0.125	0.084		-0.013	0.123		0.025
Gain per year, GS	0.124		-0.081	0.104		0.063	0.14		-0.050	0.13		0.000
Number of locations	2		2	2		1	3		3	3		3
Heritability	0.71		0.35	0.75		0.43	0.84		0.37	0.84		0.37

† CAP, CIMMYT-Asia population.

‡ GY, grain yield (t ha⁻¹).

§ ASI, anthesis silking interval (d).

of drought-tolerant source populations and inbred lines, it is important to maintain a selection pressure for reduced ASI to ensure that the ASI remains below 3 d under drought conditions.

Testcross Performance of Cycles of Selection

Grain Yield

Testcrosses of CAP1[TC-GS] and CAP2[TC-GS] were 43.1 and 3.9% better than testcrosses of CAP1[PerSe-PS] and CAP2[PerSe-PS], respectively, across the locations under drought stress (Table 4). Under optimal conditions, testcrosses of CAP1[TC-GS] and CAP2[TC-GS] were 11.4 and 33.3% better than testcrosses of CAP1[PerSe-PS] and CAP2[PerSe-PS], respectively, (Table 4) across locations. The higher response for GY under drought compared with optimal conditions is quite typical of CIMMYT germplasm. It should be noted that emphasis of CIMMYT maize breeding program has been on stable performance and resilience of improved germplasm, which lends itself to generating OPVs and hybrids that yield reasonably well compared with commercial checks under optimal conditions (years with sufficient rainfall) while showing far superior yield advantage than commercial checks under drought stress (years with scanty rainfall).

Beyene et al. (2015) used Africa-based donors to improve Africa-based recipients and reported a maximum of 24% increase in GY of C2 over C1 under drought. This is lower than what we have observed. In our study, the introgression of African drought-tolerant germplasm into an Asian germplasm base (which had no history of DT breeding) is very likely to have had very favorable complementation for DT than observations by Beyene et al. (2015). Further, in our study, it can be surmised that under optimal conditions, the complementation of African alleles with the set of alleles present in the Asian germplasm base, already adapted for optimal performance, was relatively less than that under drought. In a comparison of MARS and conventional selection, Eathington et al. (2007) reported more than double gains for MARS than conventional selection in North American and European pedigree populations. Our results

substantiated the potential of selected populations for developing drought-tolerant lines. Such lines in combinations should give hybrids resilient to drought.

Although C1 plants were selected based on marker effects of testcrosses, C2[TC-GS] population outperformed C2[PerSe-PS] for per se yield under drought. This meant that selecting for TC performance also improved the per se performance for GY, an indication of good correlation between per se and cross performance for GY under drought. Such correlation bears on the importance of additive genetic effects for GY under drought in conformation to the findings of Betrán et al. (2003).

Anthesis Silking Interval

Under drought, the reduction in ASI in C2[TC-GS] compared with the respective C2[PerSe-PS] for each population ranged from 1 to 1.4 d. Under optimal conditions, the reduction in ASI in C2[TC-GS] compared with the respective C2[PerSe-PS] for each population ranged from 0 to 1.1 d. The reduction in ASI under optimal conditions, although desirable, was of less consequence, as the highest ASI noted was 1.6 d in CAP1 C1, which was well within the optimal range for any commercial maize crop. However, any improvement in ASI under drought is of significance and in this case, an ASI of 3.3 d was noted in C2[PerSe-PS] of CAP1, which is outside the desirable range for a commercial maize crop. Under stress, and especially under drought stress, ear growth slows in relation to tassel growth and the female–male synchronization is exaggerated (ASI increases); GY and its component, ears per plant, have a high correlation with and show a dependence on ASI; ASI is a visual indicator of factors affecting reproductive success (Edmeades et al., 2000).

Discussion

The Concept

The question that we set out to answer is whether marker effects, determined from testcross performance of $F_{2:3}$ families (the training set), which when used to select plants for recombination in a later generation (Cycle 1, the test set), would (i) give a positive response to selection

Table 4. Cross performance of cycles of selection.

Generation	Drought				Optimal			
	CAP1†		CAP2		CAP1		CAP2	
	GY‡	ASI§	GY	ASI	GY	ASI	GY	ASI
Cycle 1	2.2	2.0	2.4	0.5	3.2	1.6	3.9	1.0
Cycle 2 (C2) [Per_Se-PS]	2.0	3.4	2.5	2.1	2.9	1.4	3.3	1.5
C2 [TC-GS]	2.9	1.0	2.6	1.1	3.2	1.4	4.4	0.4
Percentage improvement of C2[TC-GS] over C2[PerSe-PS]	43.1	70.6	3.9	47.6	11.4	0.0	33.3	73.3
Number of locations	1	1	1	1	3	2	3	2
Heritability	0.54	0.34	0.54	0.34	0.52	0.27	0.52	0.27

† CAP, CIMMYT-Asia population.

‡ GY, grain yield ($t\ ha^{-1}$).

§ ASI, anthesis silking interval (d).

and (ii) enable rapid development of a breeding population, which would (in per se and cross performance) be better than what is developed by a breeder through normal practice of conventional breeding.

General Practice in Developing an Improved Cycle of Selection or a Pedigree Breeding Start in Maize

Any breeder that develops an OPV, a breeding synthetic, or cycle of selection first recombines the best inbred lines selected on the basis of testcross performance. Recombination is done by bulking the pollen of a half set of the selected lines and pollinating the second half set of lines. Such a procedure ensures a random and almost complete mixing of the genetics but with loss of parental information. A more systematic approach to this would be to cross all the lines in the set in all possible combinations (diallel fashion). This is practically possible only when the number of lines in the set is manageable. This method ensures complete mixing of the genetics, enables tracking of parental information, but is more expensive to execute. Whatever the method, seeds harvested are a cross of two inbred parents (F_1) and are referred to as Synthetic 1 (Syn1).

The Syn1 generation (being an F_1) has maximum heterosis, but the seed is of finite quantity. To maintain the genetics and increase the seed, such populations are further recombined (either in isolation, pollination of demarcated sets of plants with pollen of the other set, or simply by randomly pollinating one plant with another, i.e. sib mating). This generates the Synthetic 2 (Syn2) generation (referred to as C2[PerSe-PS] in this case). Maximum inbreeding depression is seen during the formation of the Syn2, but this drastically tapers off in subsequent seed increases. In other words, negligible inbreeding depression is seen in subsequent recombinations (or seed increases). Therefore, the Syn2 is considered to be a stable and reproducible generation of the OPV, and hence, a balanced bulk of all cobs of this generation is saved as the breeder's seed.

In the process of germplasm development for abiotic stresses (like drought), selection is done for the target stress and never under the target stress. The evaluation units (units that generate data; these are either lines per se or testcrosses) are evaluated under the target stress, while the recombination and selection units (units that generate seed of the next generation) are maintained under optimal conditions. To ensure ample quantity and seed quality of the next generation, the selection units need to be increased under optimal conditions and not under the target stress (as the mean GY of an ideally stressed experiment is 20–30% of optimal yield [Bänziger et al., 2000], as tolerant genotypes can be picked only under such severe stress). Second, maintenance of uniformity of abiotic stress experiments is achieved by applying simple statistical principles of randomization, replication, and a good experimental design, possible only with separate evaluation units. Compare that with breeding

for biotic stress (disease) resistance where selection and evaluation units may be the same, as a plant identified to be resistant could give close to 100% of the normal seed yield. This procedural variation briefly defines the major difference between breeding for resistance (for biotic stresses like diseases), vis à vis breeding for tolerance (for abiotic stresses like drought).

Breeders never miss a selection opportunity; hence, during formation of the Syn2, selection may be exercised by breeders for per se plant appearance just before pollination to allow recombination of alleles of only the selected plants. Further, during mid-grain fill, pollinated plants with poor appearance (e.g., diseased plants, lodged plants, etc.) are eliminated to further decrease the frequency of unfavorable alleles. At the final step of harvest, cobs free from ear rots, desirable phenotypic appearance, good grain fill, and big cob size are selected. Note that with such a traditional maize breeding approach, a breeder has only the aforementioned visual per se criteria to enrich the Syn2 with favorable alleles in a single season. However, some breeders do not exercise this visual selection, as they prefer this as a measure of ensuring the capture of all favorable alleles and closure of the population.

Proof of Concept

Cycle 1 was formed using TC data of $F_{2,3}$ families. Cycle 2[PerSe-PS] was derived from C1 through a recombination that was based on plant per se characteristics (as described above for development of the Syn2) under optimal conditions and representing breeders' practice. Cycle 2[TC-GS] was derived by recombining C1 plants with high GEBVs for good testcross performance under drought and optimal conditions (genotype-only selection). In other words, C1 (albeit two sets) was the source population to both C2[PerSe-PS] and C2[TC-GS]. Therefore, a comparison of per se and TC performances of C2[PerSe-PS] with C2[TC-GS] gave a comparison between conventional practice followed by breeders and a molecular approach, respectively.

Both C2[PerSe-PS] and C2[TC-GS] showed improvement over the F_2 for per se and testcross performance (Table 3, 4). For reasons explained earlier, C2[PerSe-PS] was formed under optimal conditions (i.e., in the absence of the target stress—drought in this case), where the breeder is unable to pick any plants that would express DT (DT genomic regions) for recombination. For the formation of C2[TC-GS], through the use of molecular markers (and the statistically associated marker effects for combining ability under drought), the breeder is able to recombine only those plants that carry the genomic region for DT. The fact that per se performance and TC performance of C2[TC-GS] was better than C1 first validated the statistical model used for determining marker effects. Second, superior per se performance and TC performance of C2[TC-GS] compared with C2[PerSe-PS] showed that we have been able to develop a breeding population—through use of molecular markers—better than what a breeder would normally develop by

conventional practice in the absence of the target stress in one season. Cycle 2[TC-GS] was formed using marker effects of a selection index that included GY under drought (35% weightage) and optimal (40% weightage) conditions and 25% weightage for ASI under drought.

To restate this, C2[TC-GS] of both CAP1 and CAP2 showed superiority over C2[PerSe-PS] of their respective populations. Thus, use of GEBVs enabled selection of superior plant phenotypes—in the absence of the target stress—and resulted in rapid genetic gains for DT in maize. Conventional source population improvement by per se selection based on what the breeder is able to see obviously does not reveal the cross potential of the selected plants—something that markers are capable of detecting. To reveal this potential (and make use of it) through conventional selfing, testcrossing and evaluation would have taken an additional four seasons (2 yr). By having used markers in the procedure described to generate the stable source population (C2[TC-GS]), we had an opportunity to enrich it for alleles favorable for combining ability (and all in just one season). Hence, the utility of markers in picking up useful genomic regions, vis à vis breeders' practice, is validated and substantiates the potential of obtaining rapid genetic gain (per unit time) through marker applications.

Conclusions

A positive selection response can be obtained with the use of markers for GY under drought. Hence, the statistical model used for determining marker effects works in practice and thus stands validated. The use of GEBV-enabled selection of superior plant phenotypes, in the absence of the target stress, resulted in rapid genetic gains for DT in maize.

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