REVIEW & INTERPRETATION

Cross the Best with the Best, and Select the Best: HELP in Breeding Selfing Crops

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ABSTRACT

Hybrid-enabled line profiling (HELP) is a new integrated breeding strategy for self-fertilizing crops that combines existing and recently identified elements, resulting in a strategy that synergistically exceeds existing breeding concepts. Heterosis in selfing crops is often driven by additive and additive × additive gene action, the molecular basis of which is increasingly being revealed. Unlike nonadditive heterosis, additive forms can be relatively easily fixed in homozygous lines, meaning that their seed can simply be resown to express the same “heterosis.” Crossing diverse, complementary “selfing” parents to create the desired trait or allele line profile requires strict male sterility of the female; this can now be achieved relatively easily through present and emerging chemical, environmental, or genetic techniques. Fairly small amounts of hybrid seed are needed, with no need to scale up seed production, as it is not the hybrid that will be commercialized. After multilocation testing, homozygous lines from only the most superior hybrids, driven mainly by additive effects and additive × additive gene action, are rapidly derived using techniques such as doubled haploids. Multilocation testing and molecular confirmation of target line profiles then identify superior lines for release to farmers. The HELP strategy integrates modern high-throughput versions of existing and new concepts and methodologies into a breeding system strategy that focuses on the most superior crosses, <10% of all crosses. This focus results in significant increases in efficiency and can reverse the edible yield plateauing seen or feared in some of our major selfing food crops.

P L A N T breeding, certainly in the era before genomics and marker-assisted selection, was often referred to as a “numbers game.” The more crosses, the more likely it is to find improved genetic combinations. We propose a newly integrated breeding strategy for self-fertilizing crops that dramatically reduces the number of crosses being promoted while increasing the likelihood of obtaining superior new cultivars. This strategy emerges from experiments in the Bread Wheat Program of the International Center for the Improvement of Maize and Wheat (CIMMYT).

In the late 1990s we noticed that ~8% of the elite new lines in the International Bread Wheat Screening Nursery (IBWSN) for international distribution were derived from <20 crosses. The remaining 92% of elite lines came from 5000 to 10,000 crosses. These few original crosses, and the lines derived from them, represented a strategy that we now describe as hybrid-enabled line profiling (HELP). This strategy offers breeding programs for selfing crops enormous benefits through improved performance. It

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offers the potential to increase the grain yield just as $F_1$ hybrid cultivars did for cross-fertilized crops. The main reason that hybrid cultivars are so valuable is that, as a result of heterosis or hybrid vigor, they consistently perform better than their parents. For a variety of reasons, it is more difficult to take advantage of heterosis in selfing crops; the HELP strategy brings rapid and efficient selection for the benefits of heterosis to these crops.

**BACKGROUND**

The International Center for the Improvement of Maize and Wheat first started working on hybrid wheat (*Triticum aestivum*) in 1962. Since then, interest has ebbed and flowed. The original program, based on cytoplasmic male sterility (CMS), was shut down because it failed to generate sufficient heterosis (Singh et al., 2010). In 1996, with support from Monsanto, exploration started again, this time using a chemical hybridizing agent (Çukadar et al., 1997).

Belgin Çukadar led this new undertaking, producing 150 to 400 spring bread wheat hybrids each year (until 2001) in factorial mating designs. These hybrids were first yield tested near Ciudad Obregon in Mexico’s irrigated, high-grain-yield-potential environment. It rapidly became obvious that the parents of the very best hybrids also tended to be themselves high-yielding per se, as hybrid grain yield increased along with midparent and best-parent values (Çukadar et al., 2001; Çukadar and van Ginkel, 2001). We realized that according to classical genetic theory (Hallauer et al., 2010), the additive and additive × additive gene actions in this type of heterosis could be relatively easily captured in homozygous derived lines from the best hybrids, so we took $F_2$ seed from the top-yielding 5% of $F_1$ hybrids from these trials (~10 to 20 in number) to develop doubled haploids (DHs) from them.

Separate work in the program in the 1990s had shown that most directly $F_1$-derived DHs had many moderately to highly undesirable agronomic types. Hence, the $F_2$s harvested from the top hybrids entered CIMMYT’s shuttle breeding pipeline and were space planted in the highland environment of the Toluca shuttle breeding location. Seed from only the best adapted plants to this high-rainfall environment with stripe rust (*Puccinia striiformis* Westend.), Septoria, and Barley yellow dwarf virus as major pressures were planted as $F_3$s in the Ciudad Obregon shuttle breeding site, where they were selected for adaptation to its high-yield conditions, leaf rust (*Puccinia triticina* Erikss.), and stem rust (*Puccinia graminis* subsp. *graminis* Pers.:Pers.). Doubled haploids were then made from the best adapted $F_2$ plants, or the derived $F_1$ plants.

These DHs were treated in the same way as the ongoing breeding program’s $F_6$–derived $F_3$ advanced lines. The best DHs entered yield trials along with the best advanced lines through pedigree selection from the other crosses. The top yielders entered into the IBWSN.

At that point, it was noted that the only up to 20 crosses, which had been identified as superior in their $F_1$ hybrid yield trials, had spawned a disproportionate number of superior entries as DHs. Twenty-two of the 277 elite lines came from 10 to 20 hybrid crosses. Clearly, our strategy had selected the best hybrids among the 150 to 400 initial hybrids in the yield trials. We had also gained 2 yr, or four generations, in reaching this final IBWSN stage.

We took this early experience as proof-of-concept that this strategy, which we here develop further as HELP, enabled a high frequency of superior lines to be identified. Many of the conceptual insights and practical solutions that underpin HELP are rooted in advances of the past decade, especially since 2015.

**HYBRID-ENABLED LINE PROFILING (HELP)**

The HELP strategy enables the performance of $F_1$ hybrids to guide the profiling and development of $F_2$–derived lines or DHs. Such lines or DHs are the immediate source for a new cultivar emanating from HELP.

The relevant issues around the implementation of HELP are:

1. What is the physiological basis of $F_1$ heterosis, which are we aiming to capture in the derived lines, and does understanding the physiological basis help the accuracy of that process?
2. Can hybrid heterosis predict the performance of lines derived from the $F_2$?
3. What is the nature of the gene action involved in those hybrids from which superior lines could be derived?
4. What genomic tools can help to predict the extent of the additive and additive × additive gene action, based on parental and hybrid performance information?
5. How should we define the “best” parents for those “best” hybrid crosses that result in superior derived lines?
6. How can we create a high-throughput pipeline of the “best” crosses of the “best with the best” parents for widespread adoption by breeding programs?
7. How do we select the “best” hybrids?
8. How do we derive superior homozygous lines quickly from the best hybrids?

**Physiology of Heterosis**

Heterosis was first documented in a scientific fashion more than 140 yr ago by Charles Darwin (Darwin, 1876) and others. Although its physiological basis remained poorly understood, it had a huge impact on the performance of many cross-fertilizing crops. Over the past decade, however, understanding heterosis has advanced, with the prospect of extending its benefits to many more crop species. Variance components give limited knowledge regarding the different gene actions because both dominance and epistasis greatly affect additive or dominance components.
of variance. Nonetheless, as noted by Melchinger et al. (2007), additive × additive epistatic interactions, which do not contribute to inbreeding depression, appear to be a major component of midparent heterosis. Coadapted gene complexes are likely selected during inbreeding, and thus the additive × additive epistatic interactions may increase due to selection.

Several theories on the physiology of heterosis exist. Goff (2011) proposes a unifying, overarching concept of heterosis. In the context of this paper, we foremost need to know whether the physiology is determined by additive and additive × additive gene action (Sprague and Tatum, 1942), as these kinds of gene action can quite readily be captured in homozygous lines. Likewise, disomic polyploidy in durum (Triticum durum L.) and bread wheat allows alleles in their different homoeologous loci to interact as if they are homozygous in each of the respective genomes (MacKey, 1970), i.e., fixing heterosis through “overdominance” between homoeologous genomes (MacKey, 1987). Heterosis of a F1 diploid decays in its offspring (as heterozygous loci become homozygous by half at each generation), whereas pairing of homologous chromosomes in disomic polyploids as wheat does not allow intergenomic recombination, thus keeping the same heterozygosity level across generations (Comai, 2005). Furthermore, “elevated epistasis,” resulting from de novo nonallelic interactions, seems to account for genetic gains in narrow gene pools (Rasmusson and Philips, 1997). Perhaps DNA marker–aided selection among hybrid offspring after intercrossing first-generation breeding lines can lead to increasing exotic subgenomic components in further generations.

Goff (2011) explains heterosis as the result of allele-specific expression, which favors the expression of the most energy-saving, stable alleles. In hybrids, alleles at a locus are likely to be different, and allele-specific expression has multiple opportunities to express the more stable gene product. Hybrids are thus more efficient in overall energy use than lines with most loci in homozygous state and can use the saved energy for other tasks. The saved energy can be invested in greater growth rates compared with their parental lines, which we observe as heterosis.

**Hybrid Heterosis and Predicting Derived Line Performance**

Heterosis has been regularly reviewed in the scientific literature. Hence, this section deals only with research on heterosis that provides building blocks to implement the “hybrid-enabled” component of HELP.

**F₅ Performance to Predict Derived Line Performance**

Cross prediction in selfing species was first described by Jinks and coworkers in the 1970s (Jinks and Perkins, 1972; Jinks and Pooni, 1976). They assumed as target a normally distributed, quantitative trait with a mean and a variance, regardless of whether the recombinant inbred lines bearing the trait ensued from pedigree selection, single-seed descent (SSD), or DHs. The proportion expected to equal or exceed a specified target value (T) can be calculated from the normal probability integral corresponding to the standardized difference between T and \( \bar{x} \) as

\[
\frac{T - \bar{x}}{\sqrt{\sigma^2}} \text{ or } \frac{\bar{x} - T}{\sqrt{\sigma^2}}
\]

depending on whether the prediction is for higher or lower values than the target. \( \sigma^2 \) is the between-lines component of variance and equals the additive genetic component of variation, whereas \( \bar{x} \) is equal to the midparent value (m) of the two parents in the original cross, in the absence of epistasis and linkage disequilibria.

Using this approach, Hill et al. (2000) were able to identify those wheat crosses that were more likely to produce F₅ inbred lines that combined resistance to stripe rust with improved grain yield. Further diallel analysis of the F₅, following Gardner and Eberhart (1966), revealed that parental cultivar or breeding line effects and specific heterosis were the most important factors influencing grain yield in CIMMYT germplasm bred for East Africa (Ortiz et al., 2008). The significant general combining ability (GCA, cultivar or breeding line effects) and specific heterosis indicated that grain yield in this broad-based wheat breeding population was under the control of both additive and nonadditive genetic effects. Intrapopulation recurrent selection based on combining ability analysis followed by intermating between well-defined parental lines may therefore assist in capturing favorable additive and nonadditive effects to improve grain yield in wheat.

For \( \sim70 \) yr, predictions were made of the ability to extract promising homozygous lines from heterosis-expressing F₅ hybrids. These F₅s were like crystal balls, allowing one to see the upper level of performance that derived near-homozygous lines could achieve. However, when these predictions were put to the test, methodological challenges affected the outcome. In particular, the difficulty of generating sufficient F₅ seed from selfing species made it impossible to properly replicate trials in space and time. Researchers resorted to measuring grain yield on individual plants, rather than from representative multiplant plots, and doing so in a single crop season in just one location. Individual plant-based measurements have lower heritability than plot-based ones. As a result, these findings were often statistically nonsignificant and not repeatable and rarely followed up with successful practical implementation on a larger scale.

Despite these difficulties, both Matzinger (1963) and Busch et al. (1971) report derived wheat lines with performance equal to that of their original F₅s, although these were very few in number. Cregan and Busch (1977) also report superior derived F₅ lines, although because of a lack
of seed, the F₁ could not be grown each year of the trial. Thus, it proved difficult to conclusively show that advanced lines equal to their heterotic F₁s in performance can be derived in selfing crops. Furthermore, additive × additive epistatic interactions in the parents increase due to selection favoring coadapted gene complexes during inbreeding, thereby decreasing midparent heterosis over time unless the sum of dominance effects at quantitative trait loci affecting heterosis increases (Melchinger et al., 2007).

In hybrid maize (Zea mays L.) breeding, it proved more effective to first select on the F₁ testcross mean of the S₁ families of a new cross in multienvironment trials (MET) before deriving DHs from them (Longin et al., 2006). This amounts to developing homozygous offspring only from the highest performing F₁ maize hybrids, which is exactly what we wish to do in HELP for selfing crops.

**Early-Generation Bulk Populations to Predict Derived Line Performance**

Unlike F₁s in selfing crops with low seed amounts, early-generation bulks with more seed can predict good derived line performance. As early as 1940, Harlan et al. (1940) showed that the highest yielding line selections in barley (Hordeum vulgare L.) were derived from the top-yielding hybrid cross bulks. In fact, Harlan et al. (1940) stated that the low-yielding bulks could as well have been discarded from further selection without any negative impact on the program. Harrington (1940) showed similar results for wheat. In chickpea (Cicer arietinum L.), discarding poor F₁ hybrids in the first season was shown to have minimal risk, because few if any superior lines would have been derived (Byth et al., 1980). Soybean [Glycine max (L.) Merr.] has shown similar properties (Burton and Brownie, 2006; Friedrichs et al., 2016).

**Molecular Tools to Enable Heterosis to Predict Derived Line Performance**

Molecular tools have made it more feasible to use F₁ hybrids to predict the performance of derived lines. Early on, Bernardo (1994) showed that restriction fragment length polymorphism data of the parent inbred lines, augmented with how these parents did in other single crosses, made a best linear unbiased prediction of single-cross maize yields possible. Subsequent work showed that using test-cross data of inbred parental lines in hybrids, within the heterotic pattern of the two respective parental heterotic groups, allowed even more accurate prediction of the performance of new hybrid combinations of those same inbred parental lines (Bernardo, 1996). Correlations between predicted and observed grain yield were as high as \( r = 0.749 \).

The performance of F₁ hybrids can thus be used to predict that of derived lines, although the percentage of crosses from which lines exceeding the F₁ performance can be derived may be very small. Prediction can be further improved with additional information on parental line performance in related crosses.

**Additive Gene Action to Capture Heterosis in Hybrid-Derived Lines**

Heterosis is the expression of dominance deviation, the variance from midparent value, which may be explained by the additive effects of several desired dominant alleles, by “overdominance,” the combined effect of (two) different alleles at the same gene, or a combination of both (Jones, 1957). Heterosis due to dominance can be captured in homozygous individuals, as the favorable allele can be made to be present twice in homozygous lines or DHs. However, as overdominance involves different alleles at the same gene, such heterosis cannot be captured in homozygous individuals or DHs.

Recent research is increasingly showing that in selfing and some outcrossing crops, dominance is a more important form of gene action than overdominance; in other words, additive gene expression exceeds nonadditive gene action (Kaeppler, 2012; Huang et al., 2015), although Goldringer et al. (1997) found larger epistatic than additive variance for grain yield in wheat. The more the additive and additive × additive gene actions in hybrids dominate, the more effectively the F₁ performance predicts subsequent derived line performance.

The sample of published literature below gives a flavor of the understanding of gene action underpinning heterosis in three selfing crops.

**Rice**

Molecular markers indicated that additive and additive × additive gene action fuels heterosis for grain yield in rice (Oryza sativa L.) in China, up to 20% over the best parent (Xiao et al., 1995). As predicted, several derived F₁ lines yielded at least equal to the original hybrid. García et al. (2008) and He et al. (2010) showed that heterosis for grain yield and spikelets per panicle, respectively, was determined by additive and additive × additive effects. In a remarkable recent finding, Liu et al. (2015) investigated down- and upregulation of specific genes in hybrids relative to their parents, with most having additive effects (Gu et al., 2016). The downregulating of genes allowed hybrid vigor to increase. This work, to our knowledge, is the first inkling of a gene-based rationale for heterosis, opening up a new research area on how to genetically influence heterosis.

**Wheat**

Heterosis for grain yield components was observed early on and in many programs, as exemplified by a 1963 Chinese report on wheat (Chwang et al., 1963). Correlations between GCA predictions of hybrid response and actual response were medium to high in winter wheat for grain yield in France, indicative of primarily additive gene
action (Gowda et al., 2012). Heterosis for grain yield in Brazil was due to additive × additive effects, providing scope to derive outstanding lines from the best hybrids (Beche et al., 2013). Nonetheless, positive additive × additive epistatic effects seem to reduce heterosis for grain yield (Gowda et al., 2010). Although heterosis under optimum conditions has been reported many times, there is increasing interest to also identify positive heterosis for grain yield under drought. Farshadfar et al. (2013) showed that heterosis for grain yield under drought was also underpinned by high levels of additive gene action.

Barley

In an early Norwegian study heterosis in barley proved due to additive and “homozygous–homozygous” gene effects (Aastveit, 1964). F₁ lines were selected from a heterotic F₁ barley hybrid, and some lines did outyield the original F₁ hybrid. In a US study on the ratios of GCA to specific combining ability (SCA) in the F₂ and F₃ populations of two sets of barley crosses, a 5:1 advantage was found for GCA over SCA for grain yield (Smith and Lambert, 1968). Thus, additive genetic systems were mostly driving seed yield in barley, and lines exceeding their hybrids could be derived.

To summarize, lines can be derived from hybrids that equal or exceed the hybrid in performance, and such heterosis is most often associated with additive and additive × additive gene action. These gene effects, which can be captured in homozygous individuals, are cause for optimism that better selfing cultivars can be derived from those hybrids driven by additive-type gene action. The first report by Liu et al. (2015) of the actual genes driving hybrid vigor ushers in its molecular understanding.

Genomic Tools to Predict Heterosis That Can Be Captured in Derived Lines

Ideally, hybrid breeders would like to be able to predict the performance of a hybrid based on that of the parents before making the actual cross. For the HELP strategy, a second prediction is needed, which is to be able to predict the performance of derived lines based on the performance of the hybrid and its original parents.

In setting out to make new hybrids, breeders face the dilemma that there are often so many good parents available to cross that, even if assigned to different heterotic groups, the number of combinations becomes unmanageable. This is especially serious in low-investment breeding programs that lack the resources to give proper attention to each and every seemingly promising hybrid. The question then becomes: how does one focus on a limited number of just the very best crosses?

Genomic Genetic Diversity as a Tool to Predict Heterosis

Hybrid breeding dogma includes that parents need to be genetically diverse to guarantee heterosis (Boeven et al., 2016). However, genomic tools have revealed that diversity does not consistently lead to heterosis, nor underpin it as a basic genetic explanation (e.g., Martin et al., 1995; Barbosa-Neto et al., 1996; Corbellini et al., 2002 in wheat; Geleta et al., 2004 in pepper (Capsicum annuum L.); Teklewold and Becker, 2006 in mustard (Brassica carinata A. Braun); and Luo et al., 2016; Tian et al. 2016 in rapeseed (Brassica napus L.). Correlations between parental genetic distances and phenotypic hybrid performance have actually, and quite consistently, been low to very low, which shows that overall genetic diversity alone is not enough to obtain heterosis.

Genomic Analysis of Past Parental Performance in Crosses as a Tool to Predict Heterosis

Predicting heterosis focuses around using both phenotyping and genotyping data of many kinds. Based on modeling, Bernardo (1996) concluded that genotyping existing and phenotyped hybrid combinations (e.g., A/*, */B) and their parents (e.g., A and B) will allow the performance of new combinations with these parents (e.g., A/B) to be predicted, under conditions of additive gene action (Jacobson et al., 2014). In other words, if the performance of crosses between Parent A and several other parents (A/*) is known, and likewise for Parent B (B/*), then this allows prediction of the performance of the Hybrid A/B, as long as additive gene action is the major determinant of hybrid performance. Likewise, in practical pedigree breeding, it is often noted that certain parents are “good combiners,” delivering high-value crosses, which confirms the same concept. This is a major step forward in developing a hybrid prediction process and can help to keep the number of prioritized hybrids manageable. In support, interactive databases are needed to record, manage, and effectively use all phenomic and genomic information on parents in past hybrids, and that of the hybrids themselves. These data will help facilitate the prediction of new hybrid combinations with a high probability of success. We also expect the use of breeding and genetically modeled simulation to assume a more important role in such decisions (Ye and van Ginkel, 2010).

Genomic Selection as a Tool to Predict Heterosis

Genomic estimated breeding values (GEBV) for selection, or genomic selection (GS) for short (Meuwissen et al. (2001), depend on the correlation between the GEBV and the phenotype, or in other words, the relationship between genotype and phenotype. Genomic selection has proven very effective in line breeding of selfing crops (Heffner et al., 2011; Bassi et al., 2016). Rather than a
prediction of the derived phenotype per se, GEBV is the prediction of the breeding value of parents or parental contribution. That is why lines with high GEBV values should be good combiners in crosses. Can GS also contribute to the development of superior hybrids in selfing crops from which superior lines can then be derived?

According to simulation studies, GS is much more powerful than marker-assisted selection, by 18 to 43%, in selecting for quantitative traits in maize (Bernardo and Yu, 2007). Genomic selection improved grain yield and quality traits in maize much more effectively than selection using known markers with significant effects (Massman et al., 2013). It provides high levels of accuracy (i.e., the correlation between true and predicted testcross grain yield values was 0.72–0.74; Albrecht et al., 2011). Genomic best linear unbiased predictors help the “best” predicted hybrids to be identified from among the “good” hybrids, a reoccurring dilemma for breeders. Including information on the performance of both parents in other earlier crosses improves the prediction accuracy further (Technow et al., 2014). Genomic selection was especially more effective than phenotypic selection when the correlation between marker-predicted values and phenotypic values exceeded 0.50 (Krchov and Bernardo, 2015). However, apart from gain per unit cost, GS still proved advantageous at lower values in cases, where lines do not produce enough seed for actual testcrossing, and when field testing is reduced due to resource constraints. It also increased efficiency in cross prediction, especially early on in breeding programs (Kadam et al., 2016). Likewise, under drought, GS proved highly effective in identifying parental hybrid combinations that increased genetic gain in maize hybrids (Beyene et al., 2015). Genomic prediction of end-use quality traits in wheat hybrids from inbred data also proved very powerful (Liu et al., 2016a). Clearly, GS can benefit speeding up inbred and hybrid breeding, due to the ability to model between parents and hybrids (Desta and Ortiz, 2014; Zhao et al., 2015).

Metabolic and Proteomic Information Increases the Value of Genomic Selection as a Tool to Predict Heterosis

Genomic and metabolomic profiling allowed further improved accuracies for predicting GCA for complex, multigenic biomass and bioenergy traits in maize (Riedelshheimer et al., 2012). Likewise, predictability of hybrid rice grain yield was doubled when metabolomic data were added to the genomic model (Xu et al., 2016). Three multigenic traits in hybrid rice, including grain yield, could be predicted using metabolomic information from 18 parents (Dan et al., 2016). Inclusion of proteomic patterns has also been shown to be beneficial in predicting heterosis in several other crops (Xing et al., 2016). Clearly, adding metabolomic and proteomic information to the GS hybrid prediction model increases its accuracy still further.

Characterization of “Best” Parents for Hybrid-Derived Lines

Existing literature does not directly address the question of how hybrid performance can predict the performance of derived lines. However, we can extract performance information of hybrid-derived lines from the following observations on predicting hybrid performance from their parents.

The “line profiling” component in HELP comes into play at the start in deciding on the parents to be combined in the hybrid cross. At the time of initial crossing, one needs to have a good idea of what traits are desired in the profile of the final hybrid-derived lines. Specifics on the underpinning genetics of those traits will vary greatly; for some traits, entire genetic sequences and physiological and metabolic pathways are known, whereas for others, mechanisms remain unknown and we have only phenotypic descriptors.

On trait genetic aspects, for all desired line profile traits, the entire collection of potential parents must be genotyped. This includes information on GEBVs from previous crosses made with the parents under consideration, and perhaps even their ancestors. On trait physiological aspects, for all desired line profile traits, physiological trait dissection is desired. Phenomic prediction, including physiological breeding (Reynolds and Langridge, 2016; Tattaris et al., 2016), will add complementary information to genomic and metabolic information in hybrid prediction. Although relatively expensive, detailed genomic and phenomic information on the select set of superior parental inbred lines can then be used in an exponential number of hybrid combinations, including their performance prediction. This is not an extra cost to implement HELP but involves parental data generation that conventional breeding programs already have or need to acquire. We have given some examples of the gene action directing traits of the best parents in the previous sections. Additional ones are given below.

Per se Performance of Parents

In barley, per se performance of potential parents was shown to be a good predictor of a hybrid’s value in terms of superior F_{\text{s}} lines that could be derived from it (Smith and Lambert, 1968). Furthermore, GCA for grain yield was most strongly correlated with per se performance of the parents, explaining 70% of the variation in barley (Hanifi-Mekliche and Gallais, 1999). Likewise, in rice, it was concluded that “better parents” give “better hybrids,” based on per se parental performance (Tao et al., 2016). Lado et al. (2017) reach a similar conclusion for wheat, with midparent value driving genetic gain for grain yield
and contributing to cross prediction. Hence, per se performance (e.g., midparent mean) may often contribute to determining the best parents for superior hybrid combinations, but it does not provide a complete estimate.

**Parental Performance in Earlier Crosses**

At the end of the 1930s, it was already noted in maize that the highest yielding hybrids were obtained with inbred parents that had significantly contributed to other high-yielding hybrids (Hayes and Johnson, 1939). This was described as “combining ability,” but in those days, it was not yet separated into GCA and SCA. In addition, lines with good combining ability were themselves often derived from crosses between good combiners. In winter wheat, two cultivars, ‘Omar’ and ‘Selection 55-1744’, showed the highest GCA values for grain yield in a half diallel among a total of 10 genotypes (Kronstad and Foote, 1964). The authors proposed that crossing these two high-GCA cultivars would have the largest probability of resulting in high-yielding offspring, but they do not appear to have actually made the cross or conduct research on its progeny to convincingly prove their point on identifying best parents.

**Both per se Performance of Parents and Parental Performance in Earlier Crosses**

Best parents can also be determined by establishing the correlation between mean per se parental performance and mean performance of their offspring in earlier crosses (Wegenast et al., 2008). This relationship depends mostly on additive and additive × additive gene effects. Information on parental performance in earlier crosses should be available in well-organized breeding programs and is not a unique requirement for HELP. This past parental hybrid performance information will allow potential best parents to be prioritized. Dreisigacker et al. (2005) found a high correlation ($r = 0.86$) between spring bread wheat line per se yield performance and GCA, indicating that the best parents to be used in hybrids are those that have both high per se yields and strong additive gene effects. Once conveyed to the hybrids via their parent, it seems genetically likely that the same superior traits through their additive gene action can be conveyed to hybrid-derived lines.

Clearly, per se parental performance is necessary but not sufficient for superior hybrid performance. Best parents also need to have a high level of additive gene action. Crossing the best with the best parents, based on both parental per se performance and the parental record of having contributed to other good-performing hybrids through additive gene action, has a higher probability to produce outstanding hybrids, with the increased probability to spawn superior derived lines, as is the goal in HELP.

**Cross the “Best with the Best” Parents Most Effectively**

Having identified the best parents, we need high-throughput crossing procedures. However, we do not need large quantities of hybrid seed, because initial MET testing of the hybrids is limited to the major representative and predictive target production ecologies for 1 or 2 yr. Many successful breeding programs will already know what those key locations are from multilocation, multiyear genotype plus genotype × environment (GGE) biplots or similar analyses (Tadesse et al., 2013). This step of producing sufficient $F_1$ hybrid seed up front does come with additional effort and cost. Simple procedures, likely often ignored by conventional hybrid breeding programs because they do not scale to commercial levels, may well produce the relatively small amount of hybrid seed needed for HELP. Fundamentally, return on investment should drive spending, just as it does for GS or high-throughput phenotyping, which is expected to be large in the case of HELP.

Successful, “pure” cross-fertilization requires the complete absence of self-fertilization by the female parent. This is particularly important in HELP breeding because stray selfings by the female will skew the results of the various indicated phenotypic and genotypic analyses and may lead to wrong conclusions. Hence, male sterility in the female must be 100% reliable. For crops where seeds are relatively small and many are naturally borne on a plant while seeding density is relatively low, such as for certain vegetables, artificial manual emasculation of the male anthers in the female parent may, in theory, be an efficient approach, but it often remains cumbersome. Three approaches are now sufficiently advanced to implement them in high-throughput schemes, whereas several are relatively cheap and allow implementation by low-investment breeding programs. The three approaches are:

1. Apply chemical hybridizing agents prior to flowering.
2. Alter environmental cues such that pollen production is halted or interrupted.
3. Modify the genetic makeup of the plant.

**Obstructing Pollen Production Chemically**

Chemical hybridizing agents, also known as gametocides, include aliphatic acid, arsenicals, (anti-)auxins, ethephon (ethrel), and gibberellic acid (Sharma and Sharma, 2005). Modes of action include abnormal growth of the tape-latal layer, a decrease in starch production, disruption of meiosis, disruption of wall production in pollen, and non-germination of pollen. Crops that have successfully been male sterilized with chemical hybridizing agents are many and include barley, bell and chili pepper (Capsicum annuum L.), cotton (Gossypium hirsutum L.), eggplant (Solanum melongena L.), flax (Linum usitatissimum L.), groundnut (Apios americana Medik.), lettuce (Lactuca sativa L.), maize, oat
(Avena sativa L.), onion (Allium cepa L.), pearl millet [Pennisetum glaucum (L.) R. Br.], rapeseed, rice, rye, sugar beet (Beta vulgaris L.), sunflower (Helianthus annuus L.), tomato (Solanum lycopersicum L.), triticale (×Triticecale Wittm. ex A. Camus [Secale × Triticum]), and wheat (Sharma and Sharma, 2005; Prasanth and Kumary, 2014).

Obstructing Pollen Production Environmentally
Male pollen production is well known to be compromised by elevated temperatures. With climate change having gained wider attention, scientists have increased their research on plant responses to a warmer world, and some of that information can be helpful here (Hedhly, 2011; Giorno et al., 2013; Hasanuzzaman et al., 2013; Mesihovic et al., 2016). Although details vary widely, male sterility under higher temperatures is commonly noted in most of the world’s food crops, including bell pepper, carrot (Daucus carota L.), chickpea, chili pepper, common bean (Phaseolus vulgaris L.), cowpea [Vigna unguiculata (L.) Walp.], groundnut, lettuce, lima bean (Phaseolus lunatus L.), maize, okra [Abelmoschus esculentus (L.) Moench], potato (Solanum tuberosum L.), rapeseed, rice, sorghum [Sorghum bicolor (L.) Moench], soybean, tomato, and wheat. Experimenting with relatively simple procedures to elevate temperature around female flowering to induce male sterility in a semiprofessional controlled environmental chamber or greenhouse seems quite straightforward. There will be a learning curve, as this is mostly uncharted territory, but it seems certain that for many species, a controlled environmental regime can quickly be established, which will cause male sterility while maintaining female fertility. These female parents can then be fertilized with pollen from the untreated male parents to produce the necessary amount of hybrid seed.

Obstructing Pollen Production Genetically
Cytoplasmic male sterility systems can achieve 100% male sterility, whereas female fertility is maintained, and functions reliably to produce hybrid seed in some crops. However, the CMS system is complex and requires three separate parent lines to implement the system: male-sterile female lines, male (restorer) lines, and (maintainer) lines that are very similar to the female and allow seed production of the male-sterile female, as if it were self-fertilized. Many sources of literature on CMS are available and cover >150 crops (Bohra et al., 2016), although within any one crop, the genetic base for CMS is often very narrow. Global hybrid maize breeding programs dropped the CMS approach in the early 1970s, precisely because of the narrow genetic base, which resulted in disease vulnerability. In wheat, a combined photoperiod-sensitive CMS system shows promise in two-line hybrid seed production (Murai et al., 2016). We will not cover CMS further, as several alternatives appear more relevant for implementing HELP.

In hybrid rice, relatively new male sterility systems are widely used (Huang et al., 2014). Unlike three-line CMS hybrids, two-line hybrids are based on environment-sensitive genetic male sterility (EGMS) systems. The two major ones are the thermosensitive genic male sterility (TGMS) system and the photoperiod-sensitive genic male sterility (PGMS) system. In TGMS, female lines are male sterile at temperatures >28°C, allowing hybrid seed production, and male fertile at <24°C, permitting maintenance of the female line. The PGMS female lines are male sterile in short days and male fertile in long days. The genes for TGMS and PGMS have recently been identified, their functional mechanism established, and ways to rapidly convert any rice line to EGMS outlined and accomplished (Li et al., 2016; Zhou et al., 2016). Relative to three-line CMS systems, EGMS systems are simpler and have many advantages, both in breeding and in increasing genetic diversity in the program.

TGMS mutants have also been found, starting from 1948, in broccoli (Brassica oleracea L. var. botrytis L.), brussels sprout (Brassica oleracea L. var. gemmifera DC.), carrot, cotton, pepper, rapeseed, and tomato (Prasanth and Kumary, 2014), but most have yet to be investigated in depth. Given some of its seemingly universal aspects, it would appear highly feasible that EGMS-like systems can be identified and bred in other plants using the rice gene as model, with CRISPR/Cas-9 gene editing or otherwise. Recently, nanotechnology has also been proposed as a delivery system for biomolecules that affect pollen development (Wang et al., 2016) in a nontransgenic, site-targeted mode of gene editing. If these molecules are designed to be unable to pass the seed barrier, hybrid seeds will again be fully male and female fertile. Concerns about temperature fluctuations compromising pure hybrid seed set may be worthy of consideration for large field-based hybrid seed production, but for HELP, relatively small amounts of hybrid seed are the target, and small-scale local control of environmental parameters seems relatively achievable.

Select the Best F1 Hybrids
Conventional selfing-crop breeding programs select as best those advanced lines that exceed relevant commercial varieties and their top internal lines. However, in the HELP strategy, hybrids must not only exceed the best parent, but this superiority must be shown to be due mostly to additive and additive × additive gene effects, because only such gene action will be able to be captured in the subsequent derived lines. This means that full combining ability and gene action analyses must be performed on the trials. Various well-known mating designs allow combining ability and gene action to be determined, ranging from a complete diallel to more restrictive versions...
(Hallauer et al., 2010, and references therein), and any of these may be favored depending on the crop.

Based on the literature, a selection of which is cited above, and our direct in-field and laboratory experience with dozens of crop breeding programs around the world, we expect that in any selfing crop only 3 to 5% of the F₁ hybrids will meet all science- and data-based “line profiling” requisites, outlined above, to be selected as the best and promoted for derivation of lines or DHs. The corollary, therefore, is that 95 to 97% of the crosses made and evaluated as F₁s in METs need to be discarded at the end of each hybrid evaluation cycle. Likewise, based on the literature and our experience (personal communication), we assume that current breeding programs on average discard only 10 to 50% of their F₁s, doing so according to conventional criteria. Some breeders may even retain close to 100% of their F₁s, under the conviction that desirable recessive alleles will show up later. Because the HELP strategy rejects up to 97% of the F₁s, that alone increases breeding and resource-use efficiency by avoiding the planting, management, selection, and harvest of tens of thousands of subsequent ineligible segregating progenies and advanced lines over several years.

Successful, practical breeding is as much about knowing what to discard as knowing what to keep. This paper’s title, “Cross the Best with the Best, and Select the Best,” aims to stress this large initial cull in breeding programs.

**Move Quickly from the “Best” Hybrids to Derive Superior Homozygous Lines**

Various methods exist to reach homozygosity faster than simple season-by-season selfing. These include SSD (sometimes called fast generation cycling system [FGCS]), shuttle breeding, and DH. In principle, only DHs can deliver fully homozygous derivatives and do so in one generation. The other two methods deliver near-homozygous lines, which for cultivar purposes are usually acceptable, but provide additional benefits. These methods are regularly reviewed, and new modifications are often published (Choo et al., 1985; Snape, 1989; Maluszynski et al., 2003; Forster et al., 2007; Touraev et al., 2009; Tadesse et al., 2012; Dwivedi et al., 2015; Humphreys and Knox, 2015).

Independent of the method used to advance to (near-) homozygosity, this is the penultimate stage of the implementation of the “line profiling” component of HELP. Here, selected lines must meet the phenotypic (e.g., grain yield, end-use quality) and genotypic (e.g., desired allele constitution) line profiling criteria, which were to be contributed by the complementary diversity in the parents. Only those lines enter into the respective cultivar release protocol and/or are used as new superior parents in the ongoing breeding program.

**Single-Seed Descent and Fast Generation Cycling System**

In 1939, SSD was first recommended by Goulden. It has been widely used in many crops. More recently, FGCS, using SSD, produced seven generations of oat and triticale in 1 yr (Liu et al., 2016b) using stress-induced flowering and embryo rescue. In rice, using the biotron breeding system, four crossing cycles could be implemented in 1 yr (Tanaka et al., 2016). Single-seed descent and FGCS are becoming even more effective and faster, with relatively low implementation costs, making them attractive to low-investment programs.

**Shuttle Breeding**

In shuttle breeding, full-fledged selection takes place during two or more cycles per year (depending on crop cycle length), implemented in complementary selection environments. Each selection cycle in these environments contains all or most breeding generations. Thus, breeding is not only faster but also identifies more widely adapted germplasm. The time window will be small to harvest the selected entries from the closing crop cycle, process harvested seed, analyze all data, make final selection decisions, prepare seed for planting, and plant the next generations in the complementary location. Senior breeding staff themselves “shuttle” along with their crop generations from selection location to selection location. Operational costs of shuttle breeding are similar in each generation cycle, essentially multiplying the annual operational budget. This may make it prohibitive for low-investment programs, but benefits can be huge: not only is the entire breeding cycle reduced in time, but more widely adapted lines are identified.

The most well-known and oldest version of this system is CIMMYT’s wheat shuttle breeding program, established in the mid-1940s by Nobel Peace Prize laureate Norman E. Borlaug (Ortiz et al., 2007). The approach has been much replicated in many crops.

**Doubled Haploids**

Doubled haploids have been applied to rapidly achieve homozygosity in >250 plant species (Forster et al., 2015; Humphreys and Knox, 2015). By definition, DHs contain only additive genetic variation, including that arising from additive × additive epistasis, but none due to over-dominant genetic variation (Snape, 1989). As a result, DHs allow for more precise estimation of qualitative and quantitative plant traits, such as in METs, because there is no confounding within-family variation. Estimates of the speed advantage of DHs in barley are four to five fewer years (Choo et al., 1985). Doubled haploids provide additional opportunities besides speed. Gene segregation ratios are much simpler when analyzing the genetics of DHs. For example, in the case of seven segregating genes
in the F₂ of a pedigree selfing breeding program, 49,081 individual plants would need to be evaluated to find one specific homozygous genotype at a confidence level of 0.95 (Möllers and Iqbal, 2009). With DH, just 383 individual plants need be reviewed, a significant increase in efficiency. One drawback of DHs derived from the initial F₁ is that after this single recombination event, individuals carrying all desired alleles from both parents will be rare. This can be countered by additional rounds of selfing and recombination. In barley, Choo et al. (1985) recommended waiting to derive DHs until the F₂, whereas Falk (1983) in barley and Ortiz et al. (2007) in wheat advised selection in the F₂ and the F₃. If the major purpose of using DHs is to increase the speed of moving to homozygosity, then inserting two rounds of selfing will seem counterproductive. Although, the frequency of desired genotypes among the DHs would be expected to increase, this is not always the case in all crops. Sleper and Bernardo (2016) showed that DHs in maize from F₂ populations had a significant increase of ~50% in number of recombinations across the genome compared with those from F₁s, but this was not associated with greater genetic gain. Indeed, other considerations also come into play when deciding whether to include additional selfing rounds. In simulation studies, CIMMYT (Li et al., 2013) found that DHs from F₁s and F₃s in wheat did not exceed the “genetic gains per cycle, per year, and per dollar” from shuttle breeding. As a component of HELP, DHs will certainly speed up the overall strategy.

The various integrated steps involved in HELP are depicted in Fig. 1. The most “suitable” parents in terms of per se performance for all desired traits, related allele constitution, additive and additive × additive gene action, and past performance in crosses are selected for the crossing program, representing the first breeding cycle. Throughout the entire HELP process, a database is assembled, populated, updated, and consulted for next steps. In Fig. 1, the parents are indicated by color coding in ascending order of combined trait suitability, which is updated as various rounds of HELP enrich the parental database. All crosses are made that are considered able to bring all complementary alleles together, as per the final derived line profiling that is desired. This is aided by either chemical, environmental, or genetic means. These F₁ hybrids are tested in METs in the second and third breeding cycle. Only the top-performing F₁s are promoted to two selfing cycles, F₂ and F₃, involving phenotypic and genotypic selection for the desired traits. After these fourth and fifth selection cycles, homozygous progeny is obtained through either SSD, shuttle breeding, or DH. This will occupy one to three breeding cycles. The homozygous derived lines

Fig. 1. The full hybrid-enabled line profiling (HELP) cycle is depicted from assembly of most suitable parents to facilitated crossing, phenotypic and genotypic testing, facilitated derivation of lines or doubled haploids (DHs), and final testing against the initial desired line profile. One HELP round occupies ~10 breeding cycles. For additional description see text. MET, multi-environment trial; GCA, general combining ability; SCA, specific combining ability.
or DHs, now in their sixth to eighth breeding cycle, are evaluated against their initial line profiling by phenotypic and genotypic testing. By the 8th to 10th selection cycle, the best lines are identified and then submitted to the relevant cultivar release authority. One full HELP round represents ~10 crop cycles, or 5 yr if two crop cycles per year are possible, which is similar to that published for selfing crops (Wang et al., 2003).

CONCLUSION
Hybrid breeding in selfing crops has not been a major, consistent commercial success in many crops for several reasons, summarized by Longin et al. (2012). The global commercial crop area planted to hybrid cultivars of selfing crops does not even reach 1%. Nevertheless, over the past 60 yr—in national and international, public and private sectors—there has been an ebb and flow in enthusiasm for hybrids in selfing crops. The regularly observed hybrid vigor in some F1 populations, reaching 10 to 15% greater than that of the best parent, continues to arouse repeated flurries of excitement among breeders to exploit hybrid vigor. However, known obstacles tend to frustrate attempts to establish a hybrid industry for selfing crops. Rice is one of few exceptions. Based on genetic and economic considerations, wheat hybrids have been predicted to be competitive with line breeding for the near future, but not in the longer term (Longin et al., 2014). Although the future of commercial hybrids in selfing crops in their own right may be uncertain, they can be an enabler in inbred line breeding. Here, with the newly integrated HELP strategy, we show how hybrids can help identify the best performing crosses with mostly additive and additive × additive gene action, which predicts high progeny performance. Based on F1 hybrid testing, only the very top hybrids are promoted for line or DH derivation. In HELP, the F1 hybrids themselves are not the end product of commercial cultivars. Rather, hybrids help identify (near-) homozygous lines or DHs, which are then released as commercial cultivars.

The breeder’s equation refers to the selection (R), defined as

\[ R = \frac{i \sigma^2}{s_a + s_d + s_{error}} \]

where \( i \) is the intensity of selection, \( \sigma^2 \) is the additive genetic variance, and \( s_a, s_d, \) and \( s_{error} \) are the additive, nonadditive, and error standard deviations, respectively. Furthermore,

\[ R = i \frac{\sigma^2}{y (s_a + s_d + s_{error})} \]

where \( c \) and \( y \) are the parental control and number of years to complete one (recurrent) selection cycle, respectively (Ortiz, 2015). The HELP strategy’s advantage should therefore relate to \( \sigma^2 \), as well as \( c \) and \( y \), because it may seek parental control, increase selection intensity, and reduce time per cycle, thus improving accuracy and saving resources.

We recommend HELP to all breeding programs of selfing crops, small or large, public or private, low or high investment. To begin, we suggest a small HELP-based breeding program alongside your conventional programs, as we initially established at CIMMYT. This will allow unbiased comparison of the HELP-derived DHs or lines with conventionally selected inbred lines and may result in the same pleasant surprise of such disproportionately frequent superior lines that motivated this paper.

Conflict of Interest
The authors declare that there is no conflict of interest.

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