Doubled Haploids versus Conventional Breeding in CIMMYT Wheat Breeding Programs

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ABSTRACT

Doubled haploid (DH) technology has been used in breeding programs for several decades and is currently the method of choice in a number of crop species, including barley (*Hordeum vulgare* L.), rapeseed (*Brassica napus* L.), maize (*Zea mays* L.), and wheat (*Triticum aestivum* L.). In this study we investigated via computer simulation the benefit of using DHs compared with the conventional wheat breeding strategy used at CIMMYT. Two strategies using DHs were considered: DH lines directly derived from F_1 hybrids (F_1–DH), and DH lines derived from F_3 individuals that are retained following selection for agronomic traits in the F_2 generation (F_3–DH). Genetic gains per cycle, per year, and per dollar spent were consistently higher for conventional breeding than for DH breeding strategies, especially gains per dollar. Though the F_1–DH strategy saved 1 yr in completing a breeding cycle, genetic gains per year for the adaptation trait from F_1–DH were much lower than those from conventional breeding, where two growing seasons are used per year. Though the DH breeding strategy showed no significant advantages over the conventional wheat shuttle-breeding regime of CIMMYT, we did not exclude the possibility that the DH breeding strategy may have advantages when genetic gains per unit of time are considered, and only one generation is grown per year. The conventional shuttle regime will continue to be the major wheat breeding strategy at CIMMYT, where two cycles can be grown per year and breeders can do selection in large populations in both cycles.

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Abbreviations: CBs, crossing blocks; DH, doubled haploid; F_1–DH, the breeding strategy of DH lines directly derived from the F_1 hybrids; F_3–DH, the breeding strategy of DH lines derived from F_3 individuals that are retained from the selection of agronomic traits in the F_2 generation; ID, identification; MODPED, modified pedigree/bulk selection method; QuLine, a QU-GENE application breeding simulation module; QU-GENE, a simulation platform for quantitative analysis of genetic models developed by the University of Queensland (http://www.uq.edu.au/lcafs/qugene/), Australia; SELBLK, selected bulk selection method.

DOUBLED HAPLOID (DH) systems are now available in a number of crop species (Snape, 1989; Raina, 2010; Murovec and Bohanec, 2011), including barley (*Hordeum vulgare* L.), rapeseed (*Brassica napus* L.), maize (*Zea mays* L.), and wheat (*Triticum aestivum* L.) (Zheng et al., 2002; Liu et al., 2002; Melchinger et al., 2005). These systems allow completely homozygous lines to be developed from heterozygous parents in a single generation (Bruins et al., 1996; Kunz et al., 1999; Zheng et al., 2002; Forster and Thomas, 2010). In open-pollinating species, such as maize, haploid induction offers a promising alternative to recurrent selfing for rapid inbred line development (Murovec and Bohanec, 2011).

In self-pollinating species (e.g., wheat), such a system can be used to increase the efficiency of cultivar development programs (Bruins et al., 1996; Kunz et al., 1999; Liu et al., 2002; Zheng et al., 2002). Time can be saved in obtaining desired materials and there may be an increase in selection efficiency over conventional practices because of increased additive genetic variance, absence of dominance variation and within-family segregation, and decreased environmental effects through greater possibilities for replication (Forster and Thomas, 2010). Through studying DH populations, more than 130 quantitative traits have been successfully mapped in many crop species, including barley, cabbage (*Brassica capitata* var. alba L.), flax (*Linum usitatissimum*), maize, mustard (Brassicaceae), pepper (*Capsicum annuum*), rapeseed, rice (*Oryza sativa*), and wheat (Forster and Thomas 2010). In breeding, Bernardo (2009) showed that DHs should be induced from F_2 plants rather than F_1 plants to sustain long-term response. Simulation studies have also suggested that selection gains may be higher if testcrosses of F_2 plants are first evaluated in yield trials, and DHs are subsequently developed only from the F_2 plants (or their F_3 families) with the best performance (Longin et al., 2007b; Wegenast et al., 2008). Longin et al. (2006) investigated the optimum resource allocation in hybrid maize breeding with doubled haploids (DHs). In two-stage selection, they found the optimum allocation of resources involves evaluation of (i) a large number of lines in a small number of test locations in the first year and (ii) a small number of the selected superior lines in a large number of test locations in the second year. For the choice of testers, Longin et al. (2007a) determined the optimum allocation of number of lines, test locations, and the number and type of testers in hybrid maize breeding using DHs in a two-stage selection process for improvement of general combining ability and showed that testers combining a large number of divergent lines were best.

The global impact of the CIMMYT wheat improvement program has been significant and well documented (Rajaram et al., 1994; Rajaram, 1999; Ortiz et al., 2007; Ortiz et al., 2008). One main element of the program has been the shuttle breeding between two contrasting locations in Mexico (Rajaram et al., 1994; van Ginkel et al., 2002; Ortiz et al., 2007). Two cropping seasons can be grown each year: one is from November to April in Ciudad Obregón (27° N lat, 39 m above sea level), and the other is from May to October in Toluca (19° N lat, 2640 m above sea level). Therefore, 3 to 4 yr are needed to advance the breeding populations to F_6 or F_7, when the yield trials can be conducted; and 4 to 5 yr to advance to F_9 or F_{10}, when breeding materials can be distributed to national agricultural research systems of developing and developed countries. CIMMYT does not officially release cultivars, but CIMMYT's partners and collaborators release elite materials from the international trials and nurseries.

Another key factor for the success of CIMMYT's wheat breeding has been the efficient and effective breeding strategies adopted by CIMMYT breeders. Pedigree selection methods were used primarily from 1944 until 1985. From 1985 to the mid-1990s, the main selection method was a modified pedigree/bulk method (MODPED) (van Ginkel et al., 2002; Wang et al., 2003), which successfully produced many of the widely adapted wheat cultivars now being grown in the developing world. This method was replaced in the late 1990s by the selected bulk method (SELBLK) (Singh et al., 1998; van Ginkel et al., 2002) to improve resource-use efficiency. The major advantage of SELBLK compared with MODPED was that fewer seed lots needed to be harvested, threshed, and visually selected for seed appearance. In addition, there were significant savings in time, labor, and costs associated with nursery preparation, planting, and plot labeling, and potential sources of error were avoided (van Ginkel et al., 2002; Wang et al., 2003).

The DH technology has been extensively used in breeding programs worldwide (Cost Action 851, 2012) for self-pollinating species, such as wheat (Bruins et al., 1996; Kunz et al., 1999; Zheng et al., 2002; Liu et al., 2002; Mago et al., 2011), barley (Emebiri et al., 2009), and rapeseed (Custers 2003), and for cross-pollinating species, such as maize (Forster and Thomas, 2010). In breeding programs for self-pollinating species, advanced homozygous lines are the final breeding products, whereas for open-pollinating species the major objective is to improve the testcross performance as well as select superior hybrids. It is not clear whether DH technology has significant advantages if used in CIMMYT's conventional wheat shuttle-breeding scheme, where two cycles can be grown per year and selection can be conducted in both cycles. The objective of this study was to use computer simulation to investigate the benefit of using DH technology under a wide range of genetic models when compared with the SELBLK method currently used in the CIMMYT wheat breeding.

MATERIALS AND METHODS
Genotype by Environment System Used in Simulation

We used QU-GENE simulation program (The University of Queensland, 2012), in which a genotype by environment system needs to be defined; it requires information on environment types, traits, genes, the effect of genes on traits in different types of environment, and trait heritabilities (Wang et al., 2003, 2004). One environment type (i.e., no gene by environment interaction) and two traits were defined. The first trait is the so-called adaptation (trait identification [ID] is 1) and the other is yield (trait ID is 2). Adaptation is a combined index of various breeding target traits, such as maturity, plant height, yield components, and quality, which can be selected for in early generations before yield trials begin. We considered 210 additive

Genetic Model	1	2	3	4	5	6	7	8
Adaptation (A) only	100	50	0	30	0	150	60	0
Yield (Y) only	60	60	60	150	150	60	150	0
Adaptation and Yield (AY)	50	100	150	30	60	0	0	210

genes, whose effects were randomly drawn from uniform distribution U[0, 1]. Ten genes were evenly distributed on each of the 21 wheat chromosomes, and the distance between two neighboring genes was set at 10 cM.

Eight genetic models were considered on the basis of the number of adaptation, yield, and pleiotropic genes (Table 1). Yield is the most important trait in breeding. In general, selection for most other traits is expected to increase final yield, indicated by their correlations to yield (Wang et al., 2003). Pleiotropic gene effects are assumed to cause the correlation between two traits. Close genetic linkage can also give rise to a correlation between traits, which in most cases cannot be distinguished from pleiotropic effects. Among the eight genetic models considered in this study, we assumed that Models 1, 2, 3, 4, and 5 might be more realistic, though the true genetic model of adaptation, yield, and their relationship is hardly known in practice. Models 6 and 7 may be less relevant, as selection on agronomic traits in early generations always results in indirect genetic gain for yield. Model 8 represented another extreme scenario where every adaptation gene had a pleiotropic effect on yield.

Heritability in broad sense at the individual plant level was set at 0.50 for adaptation and 0.20 for yield. Nine crossing blocks (CBs), each consisting of 200 inbred parents, were generated. The nine CBs represented allele frequencies of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9, respectively. Two outcomes from running QU-GENE were used for simulating the breeding program: one was the genetic model, from which the phenotypic values of defined traits for any individual were determined and then used in selection, and the other was the initial breeding parental population from which the crosses were made.

Doubled Haploid and Conventional Breeding Strategies

QuLine (available from http://www.uq.edu.au/lcafs/qgene/ [accessed 14 Sept. 2012]) is a QU-GENE application module that simulates breeding programs for developing inbred lines. It has been used to compare two selection strategies (Wang et al., 2003), to study the effects on selection of dominance and epistasis (Wang et al., 2004), to predict cross performance using known gene information (Wang et al., 2005), to optimize marker-assisted selection to efficient pyramid multiple genes (Wang et al., 2007a), to investigate the use of identified quantitative trait loci marker associations in improving rice quality (Wang et al., 2007b), and to investigate the efficiency of a single backcross-breeding strategy in wheat breeding (Wang et al., 2009). The two output files from QU-GENE are the input files to QuLine. Besides that, breeding methods need to be clearly defined to run QuLine. One breeding method contains the crossing and selection details in each generation in an entire breeding cycle. A breeding cycle begins with crossing and ends with the generation when the selected advanced lines are returned to the CB as new parents. One breeding cycle was considered in the simulation. Thirty single crosses were made at the beginning of each breeding cycle. Only the final selected lines were used as parents for the next breeding cycle. The parents in the current CB were considered for crossing in the following cycles. Three breeding strategies were considered in the simulation. These were abbreviated as F_1–DH, F_3–DH, and SELBLK. These strategies are explained below.

Definition of the F_1–DH Breeding Strategy

Four generations were included in each breeding cycle in F_1–DH (Fig. 1). Ten F_1 individuals were generated from each hand-pollinated cross between two parents in a CB and harvested in bulk, resulting in just one family in the next generation, with "bulk" being defined as the generation advance method. In the "F_1–DH" generation, 100 DH lines from each F_1 were produced from the pollens of the 10 F_1 individuals and then harvested

```
!StrategyNumber    StrategyName        NumGenerations
 1                 F1DH                4
!NR SS  GT         PT         GA        RP PS    NL  ET...          Row 1
!                                                    AT  (ID SP SM)...  Row 2
!                                                    WT  (ID SP SM)...  Row 3
 1  0   CB         clone      bulk      1  1     1   1
                                                 0
                                                 0
 1  0   F1         singlecross bulk     1  10    1   1
                                                 0
                                                 0
 1  0   F1DH       DH         pedigree  1  100   1   1
                                                 0
                                                 0
 1  0   Increase   self       bulk      1  10    1   1
                                                 1   1 T 0.10
                                                 0
 1  0   YieldTest  self       bulk      1  100   1   1
                                                 1   2 T 0.10
                                                 0
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Figure 1. Definition of the F_1–double haploid (DH) breeding strategy in QuLine. Four generations were included in one breeding cycle in F_1–DH. Ten F_1 individuals were generated from each hand-pollinated cross between two parents in a crossing block and harvested in bulk, resulting in just one family in the next generation, with "bulk" being defined as the generation advance method. In the F_1–DH generation, 100 DH lines from each F_1 were produced from the pollens of the 10 F_1 individuals and then harvested individually, resulting in a total of 3000 DH families in the next generation, with "pedigree" being defined as the generation advance method. Each F_1–DH individual was selfed to increase the seed for adaptation and yield tests. Among-family selection was conducted for adaptation, with 1 as the trait identification (ID) in selection. Selection mode "T" and selected amount 0.1 indicated 10% of the 3000 DH families (i.e., 300) with the highest agronomic performance were selected. Each selected family was harvested in bulk. To perform the yield test, 100 plants were generated by selfing the 10 individuals in each DH family. Among-family selection was conducted on yield, with 2 as the ID in selection, and 30 DH families (i.e., 10% of the 300) with the highest yield were selected. NR, number of selection rounds; SS, seed source indicator; GT, generation title; PT, seed propagation type; GA, generation advance method; RP, number of replications for each family; PS, population size in each replication for each family; NL, number of test locations; ET, environment types for all locations; AT, number of among-family selection traits; ID, trait number; SP, selected proportion; SM, selection mode; WT, number of within-family selection traits; T, top.

individually, resulting in a total of 3000 DH families in the next generation, with "pedigree" being defined as the generation advance method. Each "F$_1$–DH" individual was selfed to increase the seeds for adaptation and yield tests. Among-family selection was conducted for adaptation, with 1 as the trait ID in selection (Fig. 1). Selection mode "T" and selected amount 0.1 indicated 10% of the 3000 DH families (i.e., 300) with the highest agronomic performance were selected. Each selected family was harvested in bulk. To do the yield test, 100 plants were generated by selfing the 10 individuals in each DH family. Among-family selection was conducted for yield, with 2 as the ID in selection (Fig. 1). Thirty DH families (i.e., 10% of 300) with the highest yield were finally selected and used to calculate the genetic gain to compare with the other two strategies.

Definition of the F$_3$–DH Breeding Strategy

Six generations were included in each breeding cycle in F$_3$–DH. Ten F$_1$ individuals were generated from each hand-pollinated cross between two parents in the CB and harvested in bulk, the same as the F$_1$ generation in the F$_1$–DH strategy (Fig. 1 and 2). One thousand F$_2$ individuals were generated for each single cross. Thirty F$_2$ individuals were selected on the basis of their high agronomic performance and harvested in bulk. By selfing the 30 selected F$_2$ individuals, 50 F$_3$ individuals were generated, from which 100 DH lines were produced, resulting in a total of 3000 DH families in the next generation. Each "F$_3$–DH" individual was selfed to increase seeds for adaptation and yield tests. Among-family selection was conducted for adaptation, and 10% of the 3000 families (i.e., 300) with the highest agronomic performance were selected. Each selected family was harvested in bulk. To perform the yield test, 10 individuals in each family were selfed, which generated 100 plants. Among-family selection was conducted for yield by selecting 30 families (i.e., 10% of the 300) with the highest yield and genetic gain calculated. We assumed that selection for some traits could not be properly conducted until the maturity stage. Therefore, DH lines could only be developed in the following F$_3$ generation.

Definition of the Selected Bulk Selection Method Breeding Strategy

Eight generations were included in each breeding cycle in SELBLK. The first three generations were the same as those in F$_3$–DH (Fig. 2 and 3), except that 500 F$_3$ individuals were generated and 50 with the highest agronomic performance were selected and selfed for the next generation. By repeated selfing, 200 F$_6$ individuals were produced from the 50 selected F$_2$ individuals. Fifty individuals with the highest agronomic performance in each F$_6$ family were selected and harvested individually, resulting in a total of 1500 families in the next generation. Ten F$_7$ individuals were grown in each family, and the 300 families with the highest agronomic performance were selected for the next generation of yield testing. Similar to F$_1$–DH and F$_3$–DH, 100 plants were grown in each F$_8$ family for yield testing. Thirty families (i.e., 10% of the 300) with the highest yield were finally selected and then used to calculate the genetic gain and compare with the two DH strategies.

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!StrategyNumber    StrategyName        NumGenerations
 2                 F3DH                6
!NR SS  GT          PT         GA        RP PS   NL  ET...           Row 1
!                                                    AT (ID SP SM)... Row 2
!                                                    WT (ID SP SM)... Row 3
 1  0   CB          clone      bulk      1  1    1   1
                                                 0
                                                 0
 1  0   F1          singlecross bulk     1  10   1   1
                                                 0
                                                 0
 1  0   F2          self       bulk      1  1000 1   1
                                                 0
                                                 1   1 T 0.03
 1  0   F3          self       bulk      1  50   1   1
                                                 0
                                                 0
 1  0   F3DH        DH         pedigree  1  100  1   1
                                                 0
                                                 0
 1  0   Increase    self       bulk      1  10   1   1
                                                 1   1 T 0.10
                                                 0
 1  0   YieldTest   self       bulk      1  100  1   1
                                                 1   2 T 0.10
                                                 0
```

Figure 2. Definition of the F$_3$–double haploid (DH) breeding strategy in QuLine. Six generations were included in one breeding cycle in F$_3$–DH. Ten F$_1$ individuals were generated from each hand-pollinated cross between two parents in a crossing block and harvested in bulk, the same as the F$_1$ generation in F$_1$–DH strategy. One thousand F$_2$ individuals were generated for each single cross and 30 F$_2$ individuals were selected by their high agronomic performance and harvested in bulk. By selfing the 30 selected F$_2$ individuals, 50 F$_3$ individuals were generated, from which 100 DH lines were produced, resulting in a total of 3000 DH families in the next generation. Each F$_3$–DH individual was selfed to increase the seed available for adaptation and yield tests. Among-family selection was conducted for adaptation, and 10% of the 3000 families (i.e., 300) with the highest agronomic performance were selected. Each selected family was harvested in bulk. To perform the yield test, 100 plants were generated by selfing the 10 individuals in each family. Among-family selection was conducted for yield, and 30 families (i.e., 10% of the 300) with the highest yield were selected. NR, number of selection rounds; SS, seed source indicator; GT, generation title; PT, seed propagation type; GA, generation advance method; RP, number of replications for each family; PS, population size in each replication for each family; NL, number of test locations; ET, environment types for all locations; AT, number of among-family selection traits; ID, trait number; SP, selected proportion; SM, selection mode; WT, number of within-family selection traits; T, top.

Time and Costs Required for the Three Breeding Strategies

On the basis of the laboratory facilities and experiences at CIMMYT, it was assumed that from pollen it took 1 yr to develop DH lines that can be used for seed increase. In the field, two seasons can be grown every year in the shuttle-breeding regime. Therefore, F$_1$–DH takes 3 yr to complete one breeding cycle, F$_3$–DH takes 4 yr, and the conventional SELBLK takes 4 yr (Table 1). We assumed that it costs US$0.20 to grow one wheat plant; US$10 to make one hand-pollinated single cross; and US$5 to develop one DH line. Therefore, F$_1$–DH would cost US$27,960 to complete one breeding cycle, F$_3$–DH would cost US$34,260, and the conventional SELBLK would cost US$21,360 (Table 2). Genetic gain per cycle was calculated by the difference on genotypic value before and after a breeding cycle for each trait. Years and costs of breeding strategies in

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!StrategyNumber    StrategyName      NumGenerations
 3                 SelBlk            8
!NR SS  GT         PT        GA      RP PS     NL ET...         Row 1
!                                              AT (ID SP SM)... Row 2
!                                              WT (ID SP SM)... Row 3
 1  0   CB         clone     bulk    1  1      1  1
                                               0
                                               0
 1  0   F1         singlecross bulk  1  10     1  1
                                               0
                                               0
 1  0   F2         self      bulk    1  1000   1  1
                                               0
                                               1  1 T 0.03
 1  0   F3         self      bulk    1  500    1  1
                                               0
                                               1  1 T 0.10
 1  0   F4         self      bulk    1  200    1  1
                                               0
                                               0
 1  0   F5         self      bulk    1  200    1  1
                                               0
                                               0
 1  0   F6         self      pedigree 1 200    1  1
                                               0
                                               1  1 T 0.25
 1  0   F7         self      bulk    1  10     1  1
                                               1  1 T 0.20
                                               0
 1  0   YieldTest  self      bulk    1  100    1  1
                                               1  2 T 0.10
                                               0
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Figure 3. Definition of conventional breeding (i.e., selected bulk breeding strategy in CIMMYT's wheat breeding programs) in QuLine. Eight generations were included in one breeding cycle of selected bulk selection method (SELBLK). The first three generations were the same as those in F_3–double haploid (DH), except that 500 F_3 individuals were generated and 50 with the highest agronomic performance were selected and selfed for the next generation. By consecutive selfing, 200 F_6 individuals were produced from the 50 selected F_2 individuals. Fifty individuals with the highest agronomic performance in each F_6 family were selected and harvested individually, resulting in a total of 1500 families in the next generation. Ten F_7 individuals were grown in each family, and 300 families with the highest agronomic performance were selected for the next generation of yield test. Similar to F_1–DH and F_3–DH, 100 plants were grown in each F_8 family for yield testing. Similar to F_1–DH and F_3–DH, 100 plants were grown in each F_8 family for yield testing, and 30 families (i.e., 10% of the 300) with the highest yield were selected. NR, number of selection rounds; SS, seed source indicator; GT, generation title; PT, seed propagation type; GA, generation advance method; RP, number of replications for each family; PS, population size in each replication for each family; NL, number of test locations; ET, environment types for all locations; AT, number of among-family selection traits; ID, trait number; SP, selected proportion; SM, selection mode; WT, number of within-family selection traits; T, top.

one cycle (Table 2) were used to determine the genetic gain per year and the genetic gain per dollar. Genetic gains per cycle, per year, and per dollar presented in this study are the means across 10,000 simulation runs.

RESULTS
Genetic Gains for the Adaptation Trait
Generally, the highest genetic gain was observed when the frequency of favorable allele was around 0.5, regardless of the genetic model, trait, or strategy (Fig. 4). Theoretically, in a random-mated breeding population, genetic gain (R) can be estimated by $R = k_p h \sqrt{V_A}$, where k_p is the selection intensity for selected proportion p, h is square root of the narrow-sense heritability, and V_A is the additive genetic variance for the trait under selection (Falconer and Mackay, 1996). Using a one-locus additive model as an example, $V_A = 2(1 - q)qa^2$ in a random-mated population, where $1 - q$ and q are the frequencies of two alleles at the locus in consideration, and a is the additive effect. Thus, $R = \sqrt{2(1-q)q} k_p ha$. Therefore, genetic gain is the highest when $q = 0.5$, given k_p, h, and a. When the allele frequency deviated from 0.1 to 0.5, genetic gain was decreased, which is expected from the above formula. Because of the influence of linkage in genetic models and random sampling for the initial parents, genetic gains were slightly higher when the frequency of favorable alleles was 0.4 rather than 0.5 for Models 1 and 4 to 8.

As far as breeding strategy goes, genetic gains under SELBLK were always the highest, regardless of genetic model, type of genetic gains, or frequency of alleles in the initial population (Fig. 4). Genetic gain per cycle for F_1–DH was lower than that for F_3–DH, because one more cycle of selection for adaptation was conducted in the F_2 generation in the F_3–DH strategy, which is consistent with the findings of Longin et al. (2007b) and Bernardo (2009).

Genetic gain per year and per dollar can be used to evaluate the time and cost efficiency of each breeding strategy. Genetic gains per year under SELBLK were higher than those under F_3–DH (Fig. 4). This is similar to genetic gains per cycle, because the time for the completion of the breeding process in both SELBLK and F_3–DH was 4 yr (Table 2). Compared with F_3–DH and SELBLK, the F_1–DH strategy saved 1 yr (Table 2), thus genetic gains per year for F_1–DH were higher than those for F_3–DH but still lower than those for SELBLK. Because of the lowest cost (i.e., US$21,360; Table 2) and the highest genetic gains per cycle for SELBLK, the advantage of using SELBLK over F_1–DH and F_3–DH was obvious when genetic gain per dollar was considered.

Under the assumption that additive effect of each gene follows a uniform distribution, more genes can generate larger genetic variance and result in greater genetic gain. In Models 1 to 3 and 6, 150 genes, including pleiotropic genes, controlled adaptation (Table 1). Thus, a similar level of genetic gains across these four models was observed (Fig. 4). When genes controlling adaptation increased to 210 in Model 8, genetic gain reached its highest compared with Models 1 to 7. Genetic gains under Model 5 were always the lowest compared with the other seven models, regardless of the type of genetic gain, breeding strategy, or the frequency of favorable alleles, because it had the least number of pleiotropic genes (Table 1).

Genetic Gains for Yield
Genetic gains for yield (Fig. 5) were much lower than those for adaptation (Fig. 4), regardless of the genetic model, the frequency of favorable alleles, or the breeding

Table 2. Costs during the breeding process for each breeding method. We assume that it takes 1 yr to develop double haploid (DH) lines from pollen that can be used for seed increase, and it costs US$0.20 to grow one wheat plant, US$10 to make one hand-pollinated single cross, and US$5 to develop one DH line. In the field, two seasons can be grown every year under the shuttle-breeding regime.

Breeding method	Generation	Number of crosses or families	Number of individuals per family	Total number of individuals	Time	Cost
					yr	US$
F_1–DH	F_1	30	10	300	0.5	360
	F_1DH	30	100	3000	1	15000
	DH, seed increase	3000	1	3000	0.5	600
	Field test	3000	10	30000	0.5	6000
	Yield trial	300	100	30000	0.5	6000
	Advanced lines	30				
	Total	6360		66300	3	27960
F_3–DH	F_1	30	10	300	0.5	360
	F_2	30	1000	30000	0.5	6000
	F_3	30	50	1500	0.5	300
	F_3DH	30	100	3000	1	15000
	DH, seed increase	3000	1	3000	0.5	600
	Field test	3000	10	30000	0.5	6000
	Yield trial	300	100	30000	0.5	6000
	Advanced lines	30				
	Total	6420		97800	4	34260
Selected bulk	F_1	30	10	300	0.5	360
	F_2	30	1000	30000	0.5	6000
	F_3	30	500	15000	0.5	3000
	F_4	30	200	6000	0.5	1200
	F_5	30	200	6000	0.5	1200
	F_6	30	200	6000	0.5	1200
	Field test	1200	10	12000	0.5	2400
	Yield trial	300	100	30000	0.5	6000
	Advanced lines	30				
	Total	1680		105300	4	21360

strategy, because the selection intensity for yield was lower than that for adaptation (Fig. 1–3). As expected, genetic gains for yield for an allele frequency around 0.5 reached the highest among nine frequencies of favorable alleles (Fig. 5), which is similar to the results in Fig. 4. Genetic gains per cycle under SELBLK were always higher compared with those under F_1–DH and F_3–DH, with gains for F_3–DH being higher than F_1–DH. Genetic gains per year under SELBLK were still higher than those under F_3–DH (Fig. 5), considering both strategies took the same amount of time to complete one breeding cycle. The breeding process of F_1–DH saved 1 yr compared with F_3–DH and SELBLK, and genetic gains per cycle under SELBLK for Models 1 to 7 were slightly higher than those under F_1–DH. Thus genetic gains per year under SELBLK for Models 1 to 7 were lower than those under F_1–DH.

Because of the lower cost of SELBLK (Table 1), genetic gains per dollar under SELBLK were much higher than those under F_1–DH and F_3–DH (Fig. 5), with gains for F_1–DH being higher than those for F_3–DH for yield.

The reason is that genetic gains per cycle under F_3–DH were slightly higher than those under F_1–DH for yield, whereas the cost for F_3–DH (i.e., US$34,260; Table 2) was higher than that for F_1–DH (i.e., US$27,960; Table 2). Across the eight genetic models, genetic gains under Model 6 were the lowest, because there were only 60 genes controlling yield in this model and more yield genes in other models. For Model 8, there were 210 pleiotropic genes and no genes specifically controlling adaptation or yield; genetic gains for yield were exactly the same as those for adaptation (Fig. 4 and 5).

DISCUSSION

In this simulation study, eight genetic models were considered with a different number of adaptation, yield, and pleiotropic genes. Nine CBs were defined by favorable allele frequencies and used as the initial breeding population to compare the DH breeding strategy (i.e., F_1–DH and F_3–DH) with the conventional wheat breeding strategy (i.e., SELBLK) used at CIMMYT. Simulation results
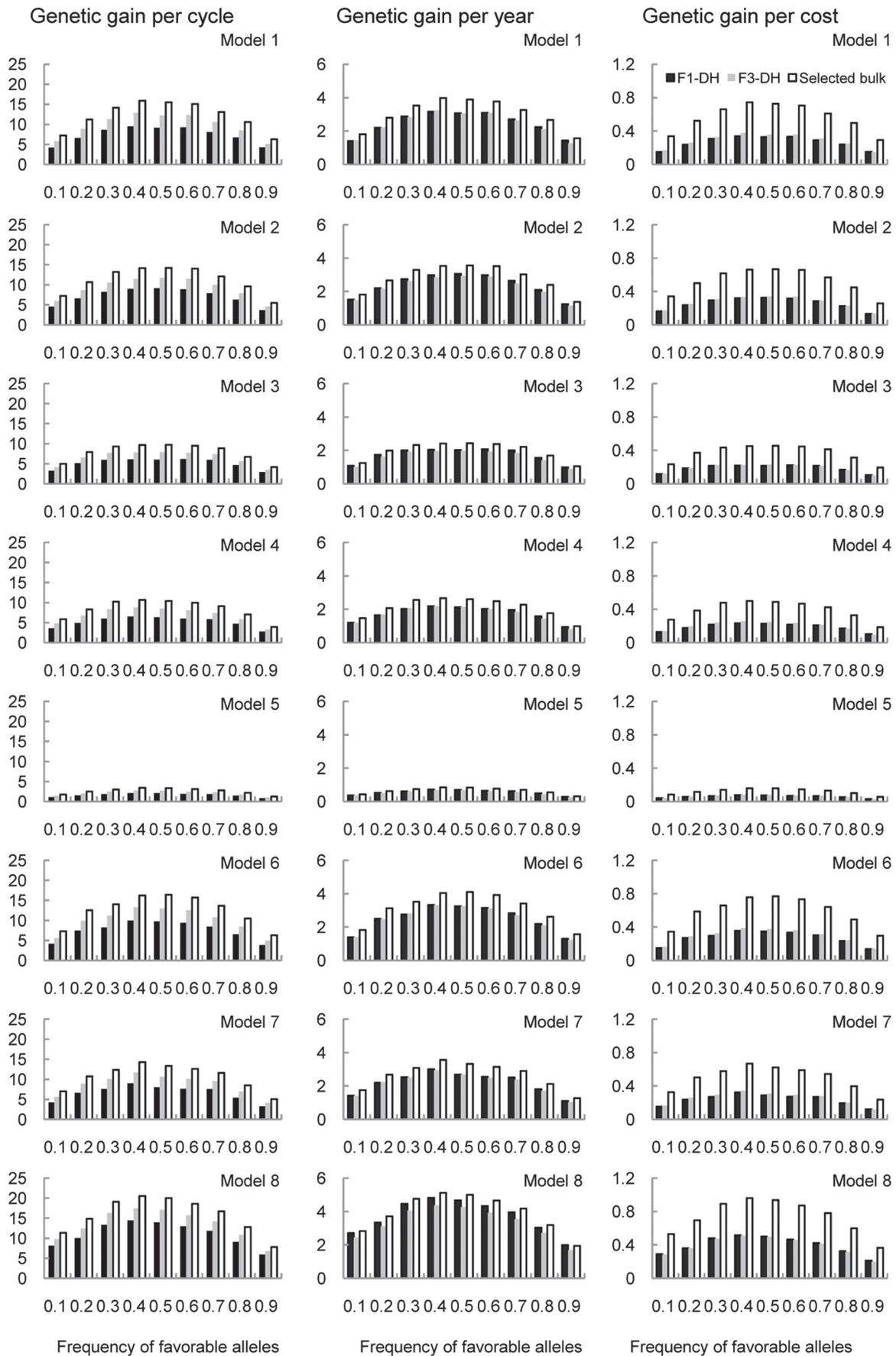
Figure 4. Genetic gains for adaptation trait under Models 1 to 8 for three breeding strategies: F_1–double haploid (DH), F_3–DH, and selected bulk selection method. Nine starting populations were considered, with the frequency of favorable alleles being 0.1 to 0.9, respectively.
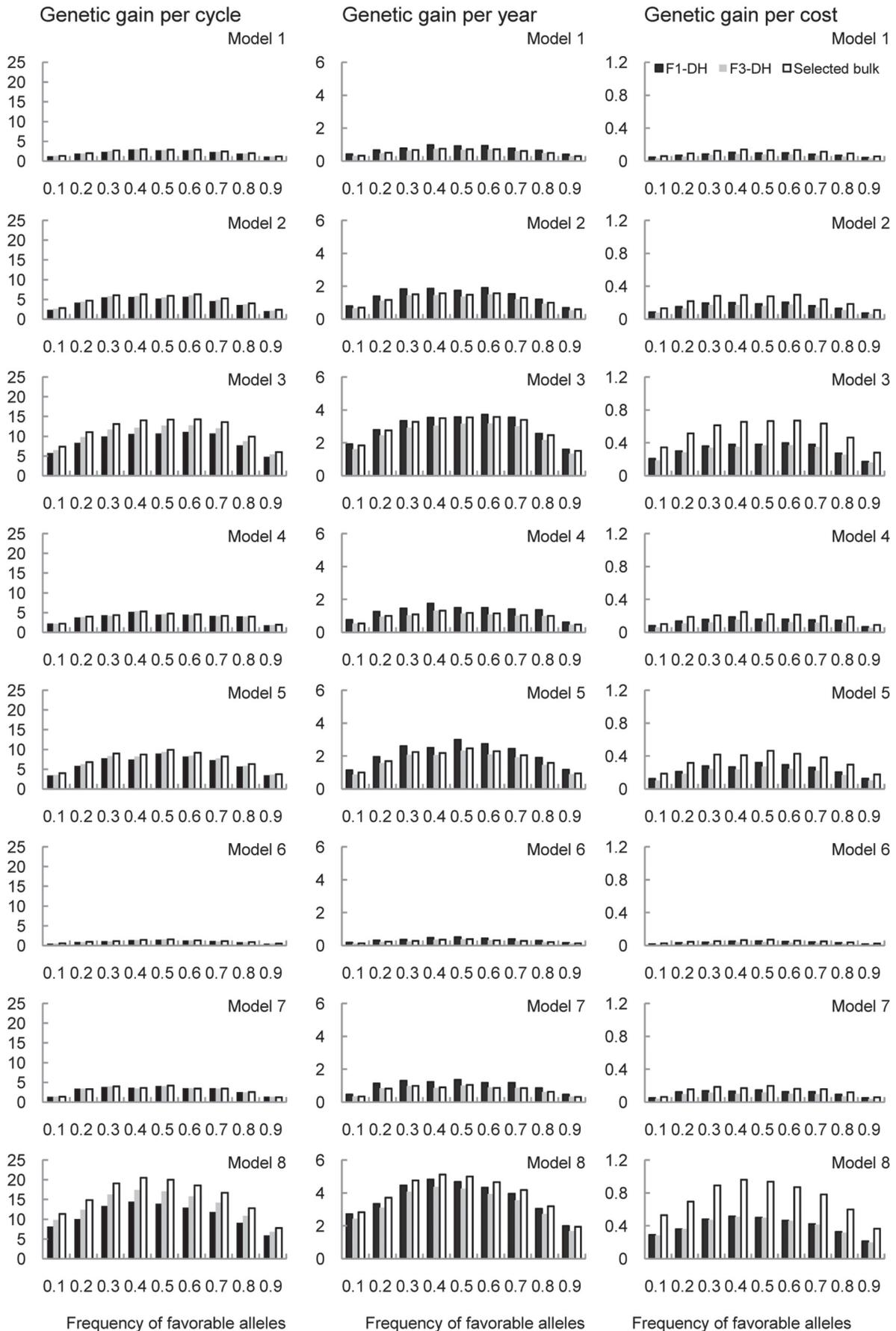
Figure 5. Genetic gains for yield trait under Models 1 to 8 for three breeding strategies: F₁–double haploid (DH), F₃–DH, and selected bulk selection method. Nine starting populations were considered, with the frequency of favorable alleles being 0.1 to 0.9, respectively.

from genetic gains per cycle, per year, and per dollar were fairly consistent. Conventional breeding methods were more efficient both genetically and economically than DH breeding strategies. For adaptation, genetic gains per year for the DH breeding strategy were lower than those for conventional breeding, even though the breeding process of the F_1–DH strategy saved 1 yr. Therefore, the DH breeding strategy showed no significant advantages over the conventional wheat breeding used in CIMMYT's shuttle-breeding approach.

For yield, genetic gains per year for the DH breeding strategy were higher than those for conventional breeding in most cases, because of the time efficiency of the DH breeding strategy. Therefore, we did not exclude the possibility that genetic gains per unit of time from a DH breeding strategy may have advantages in breeding programs where only one season can be grown every year, or where DHs can be produced in 6 mo or even less. CIMMYT wheat breeders will continue to use conventional wheat breeding, while DH technology can be used to develop some special materials for genetic studies.

The results observed in wheat seem to be contradictory to those that have been reported in maize breeding, as DH has been adopted as a routine method in many maize breeding programs (Bernardo, 1991; Seitz, 2005; Longin et al., 2006, 2007a,b). This may be understandable when we consider the different breeding objectives in maize and wheat. In maize, selection on the basis of performance of testcross is more important than that of line per se in early generations. In addition, maize F_2 populations are normally much smaller (e.g., 100–200) than those of wheat F_2 populations (e.g., 1000–3000). The development of DH is still costly and time consuming. Normally, only around 100 DH lines can be generated from an F_1 hybrid. In this sense, much stronger selection can be applied in conventional breeding, resulting in faster genetic gains. Therefore, we cannot extend these results to other crops unconditionally.

The DH method can be exploited not only to increase the efficiency in hybrid maize breeding, but also to construct genetic maps and locate genes associated with breeding traits. In fact, if selection is to be avoided as much as possible, DHs are ideal for generating genetic populations. The DH lines with completely homozygous individuals can be instantly produced so they have essentially no within-line genetic variance, whereas the genetic variance between lines is considerable, and each DH represents a different multilocus genotype (Lynch and Walsh, 1998). The DH lines can be easily bulked and assessed in multiple sites and seasons in replicated trials so that phenotype can be measured precisely, environmental variance can be controlled effectively, and genotype by environment interactions can be studied. Therefore, the lower efficiency of DHs compared with conventional breeding should not exclude the use of DH in genetic studies.

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