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QuLine, A Software that Simulates Breeding Programs for Developing Inbred Lines

Version 2.5

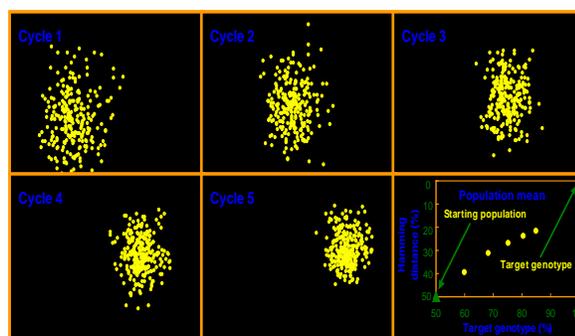
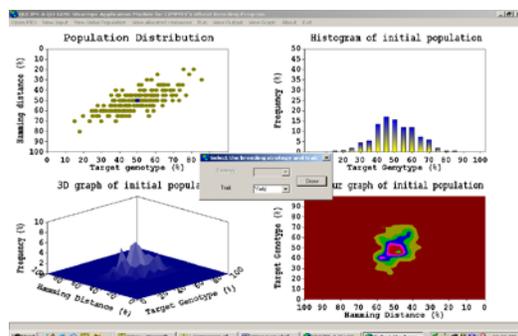
USER'S MANUAL

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1. Introduction to QU-GENE and QuLine

The major objective of plant breeding programs is to develop new genotypes that are genetically superior to those currently available for a specific target environment or a target population of environments (TPE). To achieve this objective, plant breeders employ a range of selection methods. Many field experiments have been conducted to compare the efficiencies from different breeding methods. However, due to the time and effort needed to conduct field experiments, and their associated errors, the concept of modeling and prediction have always been of interest to plant breeders.

Quantitative genetic theory generally provides much of the framework for the design and analysis of selection methods used within breeding programs. However, various assumptions are made in quantitative genetics in order to render theories mathematically or statistically tractable. Some of these assumptions can be easily tested or satisfied by certain experimental designs; others can seldom, if ever, be met, such as the assumptions of no linkage and no genotype by environment (GE) interaction. Still others, such as the presence or absence of epistasis and pleiotropy, are difficult to test. Computer simulation gives us the opportunity to lessen the impact of these assumptions, thereby establishing more valid genetic models for use in plant breeding.

Simulation as a tool has been applied in many special plant breeding studies that use relatively simple genetic models. However, a tool capable of simulating the performance of a breeding strategy for a continuum of genetic models ranging from simple to complex, embedded within a large practical breeding program including marker-assisted-selection, had not been available until recently. We introduce just such a tool here: QU-GENE, a simulation platform for quantitative analysis of genetic models that makes it possible to develop a general simulator for large breeding programs through its two-stage architecture.

The first stage involves QU-GENE as the engine, the role of which is to: (1) define the GE system (i.e., all the genetic and environmental information for the simulation experiment), and (2) generate the initial population of individuals (base germplasm) and/or a reference population to estimate genetic and/or environmental variances. The second stage involves the application modules, whose role is to investigate, analyze, or manipulate the initial population of individuals within the GE system defined by the engine. The application module usually represents the operation of a breeding

program. Within this two-stage architecture, the engine generates core information for the GE system, and the details required for specific simulation experiments are defined in the application modules. Thus simulation experiments are designed and implemented by developing an application module that interacts with information generated by the engine. Thanks to this architecture, QU-GENE has a high degree of flexibility. Numerous alternative simulation experiments that combine breeding methods with a range of GE systems can be conducted to study the performance of breeding methods under genetic models, including additive, dominance, and epistasis genetic effects, GE interaction, pleiotropy, linkage, multiple alleles, and molecular markers.

Although QuLine is a QU-GENE strategic application module developed to simulate the wheat breeding programs of the International Maize and Wheat Improvement Center (CIMMYT), the breeding strategies defined in QuLine represent the operations of most breeding programs for self-pollinated crops. Breeding methods that can be simulated by QuLine are mass selection, pedigree selection, bulk-population selection, back cross breeding, top cross (or three-way cross) breeding, doubled haploid breeding, marker-assisted selection, and any combination or modification of these methods. Simulation experiments can therefore be designed to compare the breeding efficiencies of different selection strategies, or various modifications within a selection strategy.

To run a simulation experiment in QuLine, we need:

- (1) Two software programs: the QU-GENE engine (called QUGENE) and QuLine, the application module;
- (2) Two input files: the QUG file for QUGENE and the QMP file for QuLine. There are two types of output files after running the engine: a GES file to define the GE system and a POP file to define a initial population for a breeding program. QuLine will use a combination consisting of a GES, a POP, and a QMP file as its input.

QuLine 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 (Kuchel et al. 2005; Wang et al. 2007a; Ye et al. 2007), and to investigate the use of identified QTL-marker associations and design-led breeding approaches in improving rice quality (Wang et al. 2007b).

As the number of published genes and quantitative trait loci (QTL) for various traits increases, the challenge has been and still remains to convert the ever more plentiful supply of genetic information into a large set of markers useful and breeding, and how to integrate such markers into a sustainable breeding scheme. Computer simulation allows many scenarios to be tested in silico in a relatively short amount of time, and helps breeders make decisions before conducting highly resource-demanding field experiments. QuLine can help breeders optimize the breeding strategies in conventional breeding, but also allow the investigation of the use of new technologies, such as doubled haploids, marker-assisted selection, genome-wide selection etc., improving the efficiency of conventional breeding.

2. Input file for the QU-GENE engine (QUG file)

The QUG file contains all the information required to define a genotype and environment (GE) system and the initial population to which the breeding method will be applied, which is the only input when calling the QU-GENE engine. The GE system underlies the genetic and environmental model framework for simulation experiments. In general, information about a GE system consists of some general information about the GE system, the target population of environments (TPE) for the breeding program, traits to be selected during the breeding procedure, random environmental deviations for these traits, genes for traits, their locations on chromosomes, and their effects on traits in different environment types. Information about the population consists of the number of parents and their genotypes. The sample QUG file shown in Table 1 can be used as a template for any GE system.

2.1 General information about a GE system

The first part of a QUG file is the general information about the GE system (Table 1).

- (1) *Number of models.* This parameter is specifically designed for a GE system with random gene effects. For a GE system with all gene effects (additive, dominance, epistasis, and pleiotropy) fixed, this parameter should be set at 1. The random effects model in a GE system will most likely mimic the real genetic effects of a large number of genes, such as the genes for yield. With this model some genes will have relatively larger effects and others, smaller effects. The large number of GE systems, different yield gene effects in each GE system, and replications feasible within the simulation allow many potential realities to be compared. If one breeding methodology is superior to another for all, or most, permutations, the breeder can be confident that a superior breeding methodology has been identified that is also robust to the complexities and perturbations that may emerge, regardless of the GE system.
- (2) *Random seed of random gene effects.* This random seed will ensure that the same gene effects will be assigned whenever the GE system is used, so that all random gene effects are repeatable.
- (3) *Number of genes.* Number of genes (including markers) in the GE system.
- (4) *Number of environments.* Number of environment types in TPE for the GE system.
- (5) *Number of traits.* All traits (excluding marker scores) used in selection should be counted.
- (6) *Specify names.* There are 7 indicators (a one-dimension array with size 7, each element is equal to either 0 or 1) reporting whether the names for environment types, traits, genes (loci), alleles,

epistasis networks, genotype to phenotype mapping, and populations, respectively, will be specified in the QUG file. 0 = no name specification; 1 = name specification (valid name required). Please note, for example, if the indicator for genes is set at 1, all genes should have a valid name specified in the QUG file.

2.2 Environment data

The TPE for a breeding program consists of a set of distinct, relatively homogenous environment types, each with a frequency of occurrence. Each environment type has its own gene action and interaction, providing the framework for defining GE interactions. There are three parameters for defining each environment type (Table 1):

- (1) *Row 1: Environment number.* To distinguish each environment type (arranged in order and starting from 1).
- (2) *Row 2: Name of the environment type (if defined).* If the indicator for environment type names is 1, a valid name must be specified for each environment type. If the indicator for environment type names is 0, the place is left blank.
- (3) *Row 3: Frequency of occurrence in the TPE.* Each frequency should be equal to or greater than 0.0, and the sum of all frequencies should be equal to 1.0.

Environment data are stored in the *Env(:)* array data type in the GE system definition (Fig. 2). The number of environment types can be calculated from the array size.

2.3 Trait data

For the purpose of simulation, the genotypic value of an individual can be calculated from the definition of gene actions in the GE system and from its genotypic combination. However, breeders select based on the phenotypic value in the field. Therefore, the phenotypic value of a genotype in a specific environment needs to be defined from its genotypic value and associated environmental errors. For example, if there are n plots (or replications) for a family and the plot size is m , there will be $n \times m$ individual plants (or genotypes) for this family. The genotypic value g_{ij} ($i = 1, \dots, n$; $j = 1, \dots, m$) can be defined from the GE system, and the phenotypic value p_{ij} can then be calculated using the formula $p_{ij} = g_{ij} + e_i^b + e_{ij}^w$, where e_i^b is the between-plot error for plot i , and e_{ij}^w is the within-plot

error for genotype j in plot i , and both e_{ij}^w and e_i^b are assumed to be normally distributed. The variance (σ_e^2) of e_{wij} is either user specified or calculated from the definition of heritability in the broad sense

$h_b^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$, where the genetic variance (σ_g^2) is calculated from the genotypic values of individuals

in the specified reference population in the QUG file.

In a simulation experiment, the variance of e_i^b can be set to be a proportion of σ_e^2 (i.e. $Var(e_{bi}) = r\sigma_e^2$), where the value of r is based on field data or experience. In some environments, r can be 1.5 or 2.0; in others, 1.0 or even lower. This proportion will affect the efficiency of among-plot (family) selection. Lower r means higher among-plot selection efficiency, and higher r means lower selection efficiency. Once the genotypic value of a genotype has been defined, a random effect for between-plot error from the distribution $N(0, r\sigma_e^2)$ and a random effect for within-plot error from the distribution $N(0, \sigma_e^2)$ will be added to the genotypic value g_{ij} to give the phenotypic value p_{ij} . The family mean can then be calculated from p_{ij} . QuLine then simulates within-family selection using phenotypic values and among-family selection using family means. For multiple traits, QuLine uses the independent selection process for both types of selection.

The trait data will let QuLine define the phenotypic value from the genotypic value. Therefore, the major trait information required by the QU-GENE engine is the environmental effects on traits (within-plot variance and among-plot variance) in each environment type. Either the variance or individual plant-level heritability in the broad sense needs to be specified. For heritability, the QU-GENE engine will convert the specified heritability into an estimate of environmental variance based on the provided reference population. This environmental variance is used throughout the simulation. The population structure differs from generation to generation; hence, population heritability also varies with changes in the genetic variation within the population.

There are four parameters for defining the trait data (Table 1).

- (1) *Row 1: Trait number.* To distinguish each trait.
- (2) *Row 2: Name of the trait (if defined).* If the indicator for trait names is 1, a valid name must be specified for each trait or the place is left blank.

- (3) *Row 3: Error specification type.* One-dimension array with size 3 for three kinds of error: within-plot, among-plot, and mixture-plot (not complemented yet). Two alternative values for each element:
- 1 = for the individual plant level heritability in the broad sense for within-plot; for the proportion to within-plot error variance for among-plot.
 - 2 = for error variance itself.
- (4) *Row 4+: Within-plot, among-plot and mixture-plot errors for all the defined environment types.* If error specification type is equal to 1, the value indicates heritability for within-plot, and proportion of within-plot error variance for among-plot. If error specification type is equal to 2, the specified value is for random environmental effect variance on the trait.

2.4 Gene information

Gene information is the most fundamental and complicated part of defining a GE system. It is used to generate progeny genotypes from any crossing or propagation type, and to define the genotypic value of any genotype for each trait. It consists of the location of genes on wheat chromosomes, the number of alleles for each gene locus, the number of traits affected by each gene, the genotypic effects in each defined environment type, etc. Linkage, multiple alleles, pleiotropy, epistasis, and GE interaction are all defined in this part. The following parameters define each gene (including markers) (Table 1).

- (1) *Row 1: Gene (locus) number.* To distinguish each gene (arranged in order, and starting from 1). Please note all genes should be arranged in order starting from the first chromosome or linkage group. Genes in one chromosome or linkage group should also be arranged as they appear on the chromosome.
- (2) *Row 2: Name of the gene (if defined).* If the indicator for gene name is 1, a valid name must be specified, or the place is left blank.
- (3) *Row 3: Chromosome, recombination frequency, number of alleles, and number of traits the gene affects.* All the genes, including markers, in the GE system are supposed to be arranged in order on the chromosomes. Recombination frequency of a gene is the crossover rate between the gene and the gene located just before it (two flanking genes). If a gene is located at the beginning of a chromosome, its recombination frequency should be set at 0.5.
- (4) *Row 4: Name of each allele (if defined).* If the indicator for allele names is 1, a valid name must be specified for each allele, or the place left blank.

(5) Row 5+: QTL information

- *Column 5*: Trait(s) the gene affects.
- *Column 6*: Environment type. Gene effects in all defined environments have to be specified.
- *Column 7*: Genotype to phenotype mapping (gene effect type).
 - 1 = additive and dominant gene without epistasis.
 - 2 = epistatic gene.
 - 3 = QU-GENE plug-in gene.
- *Column 8*: How gene effects are stored.
 - For additive genes (Option 1 for *Column 7*):
 - -1 = midpoint (m); additive (a) and dominance (d) will be specified later. This option is only available for genes with two alleles. For a gene with multiple alleles, option 0 should be used.
 - 0 = genotypic values in the order of AA, Aa, and aa, where A-a are the two alternative alleles on the gene locus. In case of three alleles, e.g. A1-A2-A3 at locus A, the genotypic values are arranged in the order of A1A1, A1A2, A1A3, A2A2, A2A3, and A3A3. The order is similar for more than three alleles at a gene locus.
 - 1 = random gene effects with no dominance. In the case of two alleles A and a, genotypes AA and aa have random values AA and aa ranged from 0.0 to 1.0, but the value (Aa) of genotype Aa is at the mid point between AA and aa , i.e. $Aa = \frac{1}{2}(AA + aa)$.
 - 2 = random gene effects with no over-dominance. In the case of two alleles A and a, genotypes AA, Aa and aa have random values ranged from 0.0 to 1.0, but Aa is between AA and aa .
 - 3 = random gene effects with partial/over-dominance. In the case of two alleles A and a, genotypes AA, Aa and aa have independent random

values ranged from 0.0 to 1.0, which will result in either partial dominance or over-dominance, depending on chance.

- For epistatic genes (Option 2 for *Column 7*):
 - Which epistatic network the gene is in. Genotypic values of all possible combination in an epistatic network will be defined at a later stage, once all genes in the network have been determined.
- For QU-GENE plugin genes (Option 3 for *Column 7*):
 - Which plugin the gene is in.

(6) Row 5+: Marker information

- *Column 5*: If a gene is only a marker, the trait number has to be set at 0. Trait number 0 is reserved to identify which gene locus is a marker.
- *Column 7*: Marker definition type.
 - 0 = explicit values or marker scores for marker types MM, Mm, and mm.
 - 1 = marker is dominant.
 - 2 = marker is co-dominant.
- *Column 8*: Which gene has the marker (if required).
- *Column 9*: Values for each allelic combination (if required).

2.5 Population information

For a population, the most important information is the allelic combination for each individual in the population (Fig. 3). There are basically two ways to define a population in the QUG file. One is to define the gene (or allele) frequencies in the population; then the QU-GENE engine will generate a specified number of genotypes based on the specified gene frequencies. The other is to specify the genotype of each individual in the population. In the QUG file, the number of populations to be created and the population used to estimate environmental errors (i.e., the reference population) should be given first, followed by the definition for each population. For each population the following information should be provided (Table 1):

- (1) Row 1: Population number.* To distinguish each population.

(2) *Row 2: Output file name for the population.* The extension is automatically set as POP.

(3) *Row 3: Population name (if defined).* If the indicator for population names is 1, a valid name must be specified for each population, or the place left blank.

(4) *Row 4: Population size.* Number of individuals in the population.

(5) *Row 5: Population creation type.*

- 1 = create population (alleles) (see pop1 in Table 1). The engine will assign an allelic combination to each individual in the population based on the specified gene frequencies.
- 2 = define allele frequencies only (a random seed number is required after this creation type). Only the gene frequencies are recorded for the population. The allelic combination for each individual is determined at the application stage (see pop2 in Table 1).
- 3 = specify alleles (see pop3 in Table 1). For this option all allelic combinations in the population need to be known.

For population creation types 1 and 2:

(6) *Row 6+: Information for each gene in the population.*

- *Column 1: Gene number.* If the gene number is equal to 0, all the genes have the same specifications (see pop4 in Table 1). Otherwise, all genes have to be specified.
- *Column 2: Allele order type: specify which allele at a locus will be defined first.*
 - 1 = normal order.
 - 2 = allele of interest first.
 - 3 = allele of interest under background first.
- *Column 3: Gene sampling type.*
 - 1 = population has the exact gene frequencies. In this case, the defined population will have the exactly same genes frequencies as specified to the most extent.

- 2 = population has the sampled gene frequencies. In this case, the defined population may have different frequencies from those specified, due to the sampling error.
- *Column 4: Zygosity type.*
 - 1 = both hetero-zygosity and homo-zygosity are included in the population. In the case of two alleles A and a at a locus, genotypes AA, Aa and aa have the frequencies p^2 , $2pq$ and q^2 , where p is the allele frequency of A and q is the allele frequency of a.
 - 2 = homozygous only. In the case of two alleles A and a at a locus, genotypes AA and aa have the frequencies p and q , where p is the allele frequency of A and q is the allele frequency of a.
- *Column 5: Linkage type in heterozygosity. This parameter tells how genes in double hetero-zygosity in the population, such as AaBb, are arranged on the chromosome.*
 - 1 = coupling (for AB/ab).
 - 2 = repulsive (for Ab/aB).
- *Column 6: Genotype combination manipulation type.*
 - 0 = no manipulation.
 - 1+ = genotype combination is the same as gene defined.
- *Column 7+: Allele frequency (for two alleles) or allele frequencies (for multiple alleles).* If the number of alleles in the locus is n , $n-1$ values should be specified. Frequency of the last allele is automatically determined. Please note the gene frequency should be equal or greater than 0, and the summation across all alleles at a locus should be equal to 1.

For population creation type 3, all the genotypes in the population have to be defined. The definition of each genotype is in the following order:

- *Genotype number.*
- *Genotype name.*
- *Allele combination on the first set of homologous chromosomes.*

- *Allele combination on the second set of homologous chromosomes.*

2.6 Diagnostic information

There are two diagnostic parameters at the end of a QUG file; one is for the GE system and the other is for the population. The output information will be recorded in the file whose prefix name is the same as the GE system and the extension is DGN. There are three options for each of the two parameters.

- 0 = no information output
- 1 = summary information output
- 2 = detailed information output

Table 1. Sample input file for the QU-GENE engine (Please note this is not a working example, as some information has been deleted for succinctness)

```

*** Version 2.2.01 - 1st June 2002 ***
! *****
! * QUGENE engine input file
! *****

! *** General information on the G-E system ***
! Engine G-E output filename prefix (*.ges)
test

2          ! Number of models
0          ! Random seed of random gene effects
22         ! Number of genes (includes markers and qtls)
3          ! Number of environment types
10         ! Number of traits (not including markers)
1 1 0 0 0 0 ! Specify names (ETs, Trts, Genes, Alls, EPN, GPM, pop)

! *****
! *** Environment Type Information ***
!   Row 1: Number
!   Row 2: Name (if defined)
!   Row 3: Frequency of occurrence in TPE
! *****

1
ET1
0.5000

2
ET2
0.3000

3
ET3
0.2000

! *****
! *** Trait Information ***
!   Row 1: Number

```

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```
! Row 2: Name (if defined)
! Row 3: Error Specification Type (for within,among,mixture)
!         1=heritability (spb); 2=error
! Row 4+: Within, Among, Mixture error [each ET]
! *****
```

```
1
Yield
1 1 2
  0.0500  2.0000  0.0000
  0.1000  2.0000  0.0000
  0.1200  2.0000  0.0000
```

```
2
Lodging
2 2 2
  1.5000  0.2000  0.0000
  1.7000  0.3000  0.0000
  1.9000  0.2000  0.0000
```

```
3
SR
1 2 2
  0.5000  0.5000  0.0000
  0.5000  0.5000  0.0000
  0.5000  0.0000  0.0000
```

```
4
LR
1 2 2
  0.0500  0.5000  0.0000
  0.0500  0.5000  0.0000
  0.0500  0.5000  0.0000
```

```
5
SR
1 2 2
  0.5000  1.0000  0.0000
  0.5000  0.5000  0.0000
  0.5000  0.5000  0.0000
```

```
6
Height
1 2 2
  0.5000  0.0000  0.0000
  0.5000  0.0000  0.0000
  0.5000  0.0000  0.0000
```

```
7
Tillering
1 2 2
  0.5000  0.0000  0.0000
  0.5000  0.0000  0.0000
  0.5000  0.0000  0.0000
```

```
8
Heading
1 2 2
  0.5000  0.0000  0.0000
  0.5000  0.0000  0.0000
  0.5000  0.0000  0.0000
```

```
9
GRN/SPK
1 2 2
  0.5000  0.0000  0.0000
  0.5000  0.0000  0.0000
  0.5000  0.0000  0.0000
```

```
10
TKW
1 2 2
  0.5000  0.0000  0.0000
  0.5000  0.0000  0.0000
```

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0.5000 0.0000 0.0000

```

! *****
! *** Gene Information ***
! Row 1: Number (0=same for all genes)
! Row 2: Gene name (if defined)
! Row 3: Chromosome; RF; No. of alleles; No of traits
! Row 4+: Name for each allele (if defined)
! Row 5+: (QTL)
! Column 5: Which trait
! Column 6: Environment type
! Column 7: G-P mapping type
!           1=additive gene
!           2=epistatic gene
!           3=QU-GENE plugin gene
! Column 8: Identifies how effects are stored
!           For Additive genes:
!               -1 = midpoint,additive, dominance
!               0 = explicit values (AA, Aa, aa)
!               1 = random effects with no dominance
!               2 = random effects with no over-dominance
!               3 = random effects with partial/over-dominance
!           For Epistatic genes:
!               Epistatic Network
!           For QU-GENE plugin genes:
!               Plugin mapping number
! Column 9+: Values for each allelic combination (if required)
! Row 5+: (Markers)
! Column 5: Trait 0
! Column 7: Marker definition type
!           0 = explicit values (AA, Aa, aa)
!           1 = marker is dominant
!           2 = marker is co-dominant
! Column 8: Which gene has marker (if required)
! Column 9+: Values for each allelic combination (if required)
! *****
! Columns
! 1 2 3 4 5 6 7 8 9+
! *****

1
1 0.500000 2 1
      1 1 1 0 0.70000 0.50000 0.30000
      2 1 0 1.00000 0.60000 0.00000
      3 1 -1 0.50000 0.50000 0.00000

2
1 0.050000 2 1
      1 1 1 -1 0.50000 0.50000 0.00000
      2 1 -1 0.50000 -0.50000 0.00000
      3 1 -1 0.50000 -0.50000 0.00000

3
1 0.050000 2 1
      2 1 1 0 0.30000 0.50000 0.70000
      2 1 -1 0.50000 -0.50000 0.00000
      3 1 1

4
1 0.050000 3 1
      2 1 1 0 1.00000 0.50000 0.70000 0.60000 0.50000 0.80000
      2 1 0 0.50000 0.60000 0.90000 1.10000 1.50000 1.20000
      3 1 0 1.60000 0.10000 0.30000 0.70000 0.80000 0.80000

5
1 0.050000 2 2
      2 1 1 0 0.90000 0.80000 0.70000
      2 1 0 0.70000 0.20000 0.10000
      3 1 0 0.70000 0.80000 0.60000
      3 1 1 0 2.40000 1.80000 1.20000
      2 1 0 3.20000 2.30000 1.70000
      3 1 0 1.70000 2.50000 2.60000

6
2 0.500000 2 0
      0 0 2.00000 2.00000 0.00000

7
2 0.050000 2 0

```


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```

                3   1   2
22
13 0.500000  2   1
           10  1   1   1
                2   1   2
                3   1   2

! *****
! *** Epistatic Network Information ***
!   Row 1: Number
!   Row 2: Name (if defined)
!   Row 3: Fitness Type
!           0=values specified; 1=random
!   Row 4+: Specified values (if required)
! *****

1
0
1.0  0.400  0.500  0.200  0.400  0.600  0.500  0.600  0.800

2
1

! *****
! *** Population Information (*.pop) ***
!   Row 1: Number of populations to create
!   Row 2: Which population to use for error estimates
!
!   For each population:
!   Row 1: Number
!   Row 2: Output filename prefix (*.pop)
!   Row 3: Name (if defined)
!   Row 4: Population size
!   Row 5: Population Creation Type
!           1=create population (alleles)
!           2=define allele frequencies only
!           (Row 6: Random Seed)
!           3=specify alleles
!   Row 6+:
!   Column 1: Gene (0=same for all genes)
!   Column 2: Allele order type
!           1 = normal order; 2=allele of interest first
!           3 =allele of interest (avg background) first
!   Column 3: Gene Sampling Type
!           1 = population has exact gene frequencies
!           2 = population has sampled gene frequencies
!   Column 4: Zygosity type
!           1=both hetero & homo produced; 2=homo only
!   Column 5: Linkage Disequilibrium Type of Hetero
!           1=coupling; 2=replulsion
!   Column 6: Manipulate genotype combinations type
!           0=no manipulation; 1+=genotype combinations
!           same as gene defined
!   Column 7+: Allele frequenc(ies) (No. of alleles-1)
! *****

4   ! Number of populations to create
1   ! Which population to use for error estimates

1
pop1
20
1
1  2  2  1  1  0  0.2500
2  2  2  1  1  0  0.2500
3  2  2  1  2  0  0.2500
4  2  2  1  1  0  0.2500  0.2500
5  2  2  1  1  0  0.2500
6  2  2  1  1  8  0.2500
7  2  2  1  1  8  0.2500

```

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8	2	2	1	1	0	0.2500	
9	2	2	1	1	0	0.2500	
10	2	2	1	1	0	0.2500	
11	2	2	1	1	0	0.2500	
12	2	2	1	1	0	0.2500	0.2500
13	2	2	1	1	0	0.2500	
14	2	2	1	1	0	0.2500	
15	2	2	1	1	0	0.2500	
16	2	2	1	1	0	0.2500	
17	2	2	1	1	0	0.2500	
18	2	2	1	1	0	0.2500	
19	2	2	1	1	0	0.2500	
20	2	2	1	1	0	0.2500	
21	2	2	1	1	0	0.2500	
22	2	2	1	1	0	0.2500	

2

pop2

50

2

43223

1	2	2	2	1	0	0.5000	
2	2	2	2	1	0	0.5000	
3	2	2	2	1	0	0.5000	
4	2	2	2	1	0	0.5000	0.2500
5	2	2	2	1	0	0.5000	
6	2	2	2	1	8	0.5000	
7	2	2	2	1	8	0.5000	
8	2	2	2	1	0	0.5000	
9	2	2	2	1	0	0.5000	
10	2	2	2	1	0	0.5000	
11	2	2	2	1	0	0.5000	
12	2	2	2	1	0	0.5000	0.2500
13	2	2	1	1	0	0.5000	
14	2	2	1	1	0	0.5000	
15	2	2	1	1	0	0.5000	
16	2	2	1	1	0	0.5000	
17	2	2	1	1	0	0.5000	
18	2	2	1	1	0	0.5000	
19	2	2	1	1	0	0.5000	
20	2	2	1	1	0	0.5000	
21	2	2	1	1	0	0.5000	
22	2	2	1	1	0	0.5000	

3

pop3

5

3

1

P1

11231 22111 22 2 1 2 2 1 1 1 2 2 2
 11231 22111 22 2 1 2 2 1 1 1 2 2 2

2

P2

21212 21121 13 1 1 2 1 1 2 1 2 1 2
 21212 21121 13 1 1 2 1 1 2 1 2 1 2

3

P3

12211 21112 11 2 2 1 1 2 2 2 2 2 2
 12211 21112 11 2 2 1 1 2 2 2 2 2 2

4

P4

12111 11111 22 2 2 2 1 1 2 1 1 2 2
 12111 11111 22 2 2 2 1 1 2 1 1 2 2

5

P5

21221 22111 22 2 1 2 2 1 2 2 2 1 1
 21221 22111 22 2 1 2 2 1 2 2 2 1 1

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```
4
pop4
60
1
0 2 2 1 1 0 0.2500
```

```
! *****
! *** Diagnostic Information (*.dgn) ***
! Column 1: G-E System
! 0=no information is output
! 1=summary information output
! 2=detailed information output
! Column 2: Population
! 0=no information output
! 1=summary information output
! 2=detailed information output
2 1
```

3. General information for a simulation experiment and definition of breeding strategies to be simulated by QuLine

The data structure for defining breeding strategies in QuLine is shown in Fig. 1. In this section CIMMYT's wheat breeding program will be used as an example where appropriate (Fig. 2; Fig. 3; Table 2). Other breeding methods for self-pollinated crops, such as mass selection, family selection in S1, family selection in S2, traditional pedigree system, modified pedigree, bulk population breeding, doubled haploid in F2, doubled haploid in F3, are given in Table 3.

3.1 General information for a simulation experiment

Fourteen parameters are included as general information for a simulation study.

- (1) *Number of strategies.* Number of breeding strategies to be simulated or compared.
- (2) *Number of runs.* This is the number of replications for each model in the GE system. The number of models is 1 if there is no random gene effect in the GE system. **Please note that for each the same single crosses will be made for the first breeding cycle for all strategies in all runs. Another set of single crosses will be made for another model.**
- (3) *Number of cycles.* See section 3.2 for the detailed explanation of a breeding cycle.
- (4) *Number of crosses.* The same crosses will be made for all simulated strategies in the first breeding cycle, so that the strategies can be properly compared.
- (5) *Indicator for crossing block update.*
 - 0 = only the final selected lines will be used as the parents for next breeding cycle. The parents in the current crossing block will not be considered for crossing in the following cycles.
 - 1 = for the next cycle, some parents come from the current crossing block, and some from the final selected lines. For example there are 100 parents in the starting breeding population, and 50 advanced lines are selected at the end of a breeding cycle. QuLine will first merge the final selected population with the starting population to form a new population of size 150. Then 100 (equal to the size of the initial population) individuals will be randomly drawn out of the 150 and used as the parents for the next breeding cycle.

(6) *Indicator for GE system output.*

- 0 = No output of GE system information.
- 1 = Output the GE system information model by model.

(7) *Indicator for population output.*

- 0 = No output.
- 1 = Output information about the population before selection and the population after each cycle of selection, including genotypes, genotypic values, gene frequencies, Hamming distance, genetic variances, etc. The output files with extension name .pop have the exactly same format as those from running the QU-GENE engine, and therefore can be used as the populations in other simulations.

(8) *Indicator for selection history output.*

- 0 = No output.
- 1 = Output the selection history of each line in the final selection population after each breeding cycle.

(9) *Indicator for Rogers distance output.*

- 0 = No output.
- 1 = Output the Rogers distance for each cross, the lines from each cross after each round of selection, and lines above the mid-parent and line above the better parent at the end of a breeding cycle.

(10) *Indicator for correlation output.*

- 0 = No output.
- 1 = Output the correlation coefficients among traits in each environment type and among environment types for each trait in the initial and selected populations.

(11) *Indicator for genetic variance components output.*

- 0 = No output.
- 1 = Output the genetic variance components in each environment type and among environment types for each trait in the initial and selected populations.

(12) Parents for all crosses.

- If 'random' is specified, no information for parents for all crosses is required. The parents for each cross will be randomly specified in QuLine.
- Otherwise, the input will be the name of an existing external file containing the parents for each cross. Six parents are needed for each cross, i.e., the two parents for the single crossing, the backcrossing parent, topcrossing parent, and the last two parents for double crossing.

(13) Indicator for random mating in the final selected population.

- 0 for no.
- Otherwise, the times of random mating in the final selected population.

(14) Population size of the randomly mated population.

- When random mating is needed, this parameter specifies the population size after each cycle of random mating. For the next breeding cycle, the randomly mated population will be used as the initial breeding population.

The four nested loops in QuLine should be introduced here to clarify the difference between the number of models in the GE system and the number of runs in the simulation experiment. The first layer loop is the loop for all models. Within this loop, the parents for all crosses in the first breeding cycle are determined. The next loops are all strategies, for all runs, and all cycles. So the difference among runs reflects the difference among breeding strategies, as all the strategies start from the same point, i.e., the same crossing block (initial population) and the same crosses.

3.2 Definition of breeding strategies to be simulated

A breeding strategy (Fig. 3; Table 2; Table 3) is defined in QuLine as all the crossing, seed propagation and selection activities, and experimental designs in an entire breeding cycle. A breeding cycle begins with crossing and ends at the generation when the selected advanced lines are returned to the crossing block as new parents. Most breeding programs of self-pollinated crops can be described approximately as shown in Fig. 3. This figure demonstrates the germplasm flow from making single crosses in CIMMYT's bread wheat breeding program. The selection method described in Fig. 3 is called selected bulk at CIMMYT and will be used as an example to demonstrate how a breeding

strategy can be defined in QuLine. It should be noted that variations of this selection approach are commonly used in breeding programs for self-pollinated crops.

Several breeding strategies can be defined simultaneously in one input file (QMP file) (see data type *Strategies* in Fig. 1). The QuLine module then makes the same crosses for all the defined strategies in the first breeding cycle. Hence, all strategies start from the same point (the same crossing block, the same crosses and the same GE system). All the strategies have to be defined. For each breeding strategy, the following information in sections 3.2 to 3.4 is required.

3.3 Definition of each breeding strategy

Each breeding strategy needs to be defined separately. The definition of a strategy consists of the following three parts:

- (1) *Strategy title*; name of the breeding/selection method.
- (2) *Number of generations in the strategy*.
- (3) *Definition of each generation*.

In the breeding program shown in Fig. 3, the best advanced lines developed from the F10 generation will be returned to the crossing block to be used for new crosses; i.e., a new breeding cycle starts after F10. Therefore the number of generations in one breeding cycle is 10 for the selected bulk breeding strategy. Each of the 10 generations, along with the crossing block (viewed as F0), needs to be defined (Fig. 1).

There are three key Mexican locations involved in this breeding program: Ciudad Obregon (27° N, 39 m above sea level [masl]), Toluca (19° N, 2640 masl), and El Batan (19°N, 2300 masl) (Fig. 2). Ciudad Obregon is an arid, irrigated location, and growing season conditions are similar to many other irrigated environments around the world. Yield trials for materials targeted to low rainfall and irrigated areas are conducted in Ciudad Obregon, where high yields are obtained (8-11 t/ha) and the main diseases are leaf rust (*Puccinia recondita*) and stem rust (*Puccinia graminis*). Precipitation in Toluca is high (about 800 mm during the summer crop cycle), providing favorable conditions for foliar diseases, in particular stripe rust (*Puccinia striiformis*) and foliar blights, such as *Septoria tritici*. Precipitation at CIMMYT Headquarters, El Batan, is more erratic, with an annual average of 500-600 mm; irrigation facilities are available when needed. El Batan is used mainly for leaf rust screening and

small-scale seed increases. Two crop cycles can be grown each year: November – April in Ciudad Obregon, and May – October in Toluca and/or El Batan. These environment types and their frequencies of occurrence in a specific breeding program should be defined in a GES file.

3.4 Definition of each generation

The parameters to define a generation consist of the number of selection rounds in the generation, the indicator for seed source (explained below), and planting and selection details for each selection round (Tables 2 and 3; Fig. 4). Most generations in Fig. 5 have just one selection round (e.g., F1 to F6), while some generations have more than one (e.g., F7, F8 and F9). Take the F7 as an example: once an advanced line is selected from among F7 head-rows, the F8 seed lot is split three ways. A reserve is kept for sowing yield trials at Ciudad Obregon in the following winter season [F8(YT)]; of the other two sets, one is sown at Toluca [F8(T)] and another at El Batan [F8(B)] during the summer to evaluate response to the respective disease spectra in the field. The entries composing the yield trial in the F8 generation [F8(YT)] in Ciudad Obregon are only those entries that have been proven resistant to the diseases at the two summer locations [F8(T) and F8(B)]. The F7 generation is therefore subjected to four rounds of selection: one among F7 lines, two based on disease evaluation in the field on progeny (F8) in both Toluca and El Batan, and one based on F8 yield performance in Ciudad Obregon. Since seed of the two F8 field disease evaluations and the F8 yield trial is derived from selected F7 lines (Fig. 5), the indicator of the seed source is 0 for F7 (Table 2). In other selection procedures the seed for selection round i ($i > 1$) may come from plants selected from round $i-1$. In that case the indicator for seed source is denoted as 1. For those generations subjected to just one round of selection, no indicator for the seed source is required (Table 2).

The simulation module was developed such that the key components of the field breeding process were retained. This is important firstly for the integrity of the simulation, but also to allow the breeder to retain confidence in the value of the simulation process. In practice, the F8 field disease evaluation tests in Toluca (F8(T)) and El Batan (F8(B)) are grown in the same year as the F8 yield trial (F8(YT)); the F8 small plot evaluation (F8(SP)) in Ciudad Obregon is grown just six months later (Fig. 5). Using the starting conditions and selection intensities outlined in Table 3, there would be 14,760 F7 lines in the field derived from pedigree selection in F6. Following F7 selection, 3,868 are promoted to F8 disease evaluation in the field in Toluca and El Batan. Following the F8 disease evaluation in Toluca, 2,163 F7 lines are retained; the rest are discarded. Only the 2,163 families are

considered for selection in the F8 disease evaluation in El Batan. After the F8 disease evaluation in El Batan, the among-family selected proportion is 0.90 (Table 3), resulting in the retention of 1,947 F8 families. The reserve seed of the corresponding 1,947 F7 lines is then planted simultaneously as an F8 yield trial and small plot evaluation in Ciudad Obregon. For the purposes of simulation, the four selection rounds are applied sequentially. So QuLine will first conduct F7 among-line selection in Ciudad Obregon, then the F8 field disease evaluation in Toluca, then the F8 disease evaluation in El Batan, and finally the F8 yield trial in Ciudad Obregon (Table 2). This sequential modification does not alter the essential nature of the selection process.

3.5 Definition of each selection round

The parameters that define each selection round can be found in Fig. 4.

(1) *Title of the round (or the generation).*

(2) *Seed propagation type for the selection round.*

Seed propagation type describes how the selected plants in each family selected from the previous selection round or generation are propagated to generate the seed for the current selection round or generation. The *Seed propagation type* can only be either “random” or “self” for generation 0 (i.e. crossing block), and can only be “singlecross” for generation 1. Here are the seven options for seed propagation type for any other generations, in order of increasing diversity:

- *clone*: asexual reproduction.
- *DH*: doubled haploid.
- *self*: self-pollination.
- *backcross*: backcross to one of the two parents.
- *topcross*: cross to a third parent.
- *doublecross*: cross to another F_1 .
- *random*: random mating among the plants in a family.
- *noself*: random mating but self-pollination is eliminated.

The seed for F_1 is derived from crosses among parents in the initial population (or crossing block). The female and male parents for each cross are randomly determined by QuLine if General

Information for a Simulation Experiment item (*12*) *Parents for all crosses* is specified as “random”. One may also select preferred parents from the crossing block. The selection criteria for such preferred parents, grouped in male and female master lists, can be defined in among-family selection and within-family selection (see below for details) for the crossing block (referred to as the F0 generation). In this case, crosses are made only between the female master parents and male master parents.

By using the parameter of seed propagation type, numerous ways of propagating seed can be simulated in the QuLine.

(3) *Generation advance method for each selection round.*

Generation advance method describes how the selected plants within a family are harvested. There are two options for this parameter:

- *pedigree*: the selected plants are harvested individually, and therefore each selected plant will result in a separate family in the next generation; if several plants are harvested, there will be several families in the next generation.
- *bulk*: the selected plants in a family are harvested in bulk, resulting in just one family in the next generation. Bulk happens for each family. There is no mixing of all families.
- *superbulk*: No matter the number of families, all selected individuals in all families will be mixed as one population. This option is not common in breeding, but may be used for some special genetic studies.

In the classic pedigree breeding method, *bulk* is used in F1, and *pedigree* is used from F2 to F6 or F7, while in the classic bulk breeding method, *bulk* is used from F1 to F5 or F6, and *pedigree* is used in F6 or F7.

This parameter and the seed propagation type allow QuLine to simulate not only traditional breeding methods such as pedigree breeding and bulk-population breeding, but also many combinations of different breeding methods (e.g., pedigree selection until the F4 and then DH production of the best F4 plants). The *bulk* generation advance method will not change the number of families in the following generation if no among-family selection is applied in the current generation, while the *pedigree* method increases the number of families rapidly if there is weak among-family selection intensity, and several plants are selected within

families. For a generation with more than one selection round, the generation advance method for the first selection round can be either *pedigree* or *bulk*. Subsequent rounds are used to determine which families derived from the first selection round will be advanced to the next generation. In most cases, *bulk* generation advance is preferred for subsequent selection rounds.

(4) *Field experiment design for each selection round.*

The parameters used to define the field experiment design in each selection round include:

- *The number of replications for each family.*
- *The number of individual plants in each replication.*
- *The number of test locations.*
- *The environment type for each test location.* All locations have to be specified. The value for environment type should be consistent with that defined in the GE system. Nevertheless, it can have value 0. In this instance, QuLine will randomly assign environment types to test locations with value 0 based on the frequencies of environment types in the TPE. This specification is useful where the environmental types of test locations are little understood, and a number of environment types has been defined in the GE system to simulate the effects of genotype by environment interactions.

(5) *Among-family selection and within-family selection for each selection round.*

The same data structure was used to define among-family selection and within-family selection in QuLine (Fig. 4). Please note the among-family selection in the starting population is used to selected female master, and the within-family selection in the starting population is used to selected male master. When crosses are made randomly, one parent is from the female master list, and one parent is from the male master list. The following description applies for all breeding populations, except the starting parental population.

- *Number of traits used for selection.*
- *The definition of each trait.*
 - *Trait number:* Trait number has to be in the range defined in the QUG file.
 - *Selection mode.* There are eight options for trait selection mode:

- *T for top*: individuals or families with the highest phenotypic values for the selected trait (e.g., yield, tillering, grains per spike, and thousand kernel weight) will be selected (Table 2).
 - *B for bottom*: individuals or families with the lowest phenotypic values for the selected trait (e.g., lodging, stem rust, leaf rust, and stripe rust severity) will be selected (Table 2).
 - *M for middle*: individuals or families with intermediate (middle) phenotypic values for the selected trait (e.g., height and heading) will be selected (Table 2).
 - *R for random*: individuals or families will be randomly selected (Table 2).
 - *TV for top value*: individuals or families with the phenotypic values higher than *TV* for the selected trait will be selected.
 - *BV for bottom value*: individuals or families with the phenotypic values lower than *BV* for the selected trait will be selected.
 - *TN for top number to be selected*: a specified number of individuals or families at the top (highest phenotypic values) will be selected.
 - *BN for bottom number to be selected*: a specified number of individuals or families at the bottom (lowest phenotypic values) will be selected.
 - *RN for a number of individuals or families to be randomly selected*: a specified number of individuals or families (lowest phenotypic values) will be randomly selected.
- *Selected proportion or truncation value or selected number depending on selection mode.*
- For selection mode T, B, M, and R, this will be the proportion of individuals in a family (for within-family selection) or of families in a generation (for among-family selection) to be selected.
 - For selection mode TV and BV, this will be the threshold phenotypic value to selected individuals in a family (for within-family selection) or of families in a generation (for among-family selection).

- For selection mode TN, BN and RN, this will be the number of individuals in a family (for within-family selection) or of families in a generation (for among-family selection) to be selected.

Ten traits have been included in the selection process in the breeding program in Fig. 3, but not all of them are used during selection in every round and every generation (Table 2). Among-family selection and within-family selection are distinct processes in a breeding strategy. However, these two kinds of selection have the same structure, i.e., the traits to be used in selection and their definitions (Fig. 1). Traits are given a sequential numerical code, starting with one. If a marker score is to be used for selection, the trait code given is 0. This separate classification, plus related GE system implications, allow QuLine to simulate marker-assisted selection. Independent culling will be used if multiple traits are involved in the selection. If there is no among-family or within-family selection in a selection round, the number of selected traits is noted as 0 (Table 3). In generations from F1 to F6 in this sample breeding program, each family is derived from one cross, given that the method applied is *bulk* generation advance. Among-family selection from F1 to F6 is in fact among-cross selection. Traits for both among-family and within-family selection can be the same or different, as is the case for selected proportions (Table 3). Traits for selection also differ from generation to generation, as do the selected proportions for traits.

Among-family selection in generation 0 (CB in Table 3) is used to select the female master list, and within-family selection is used to select the male master list. In Table 3, there is no trait for both among-family and within-family selections for CB, so all parents are used in crossing.

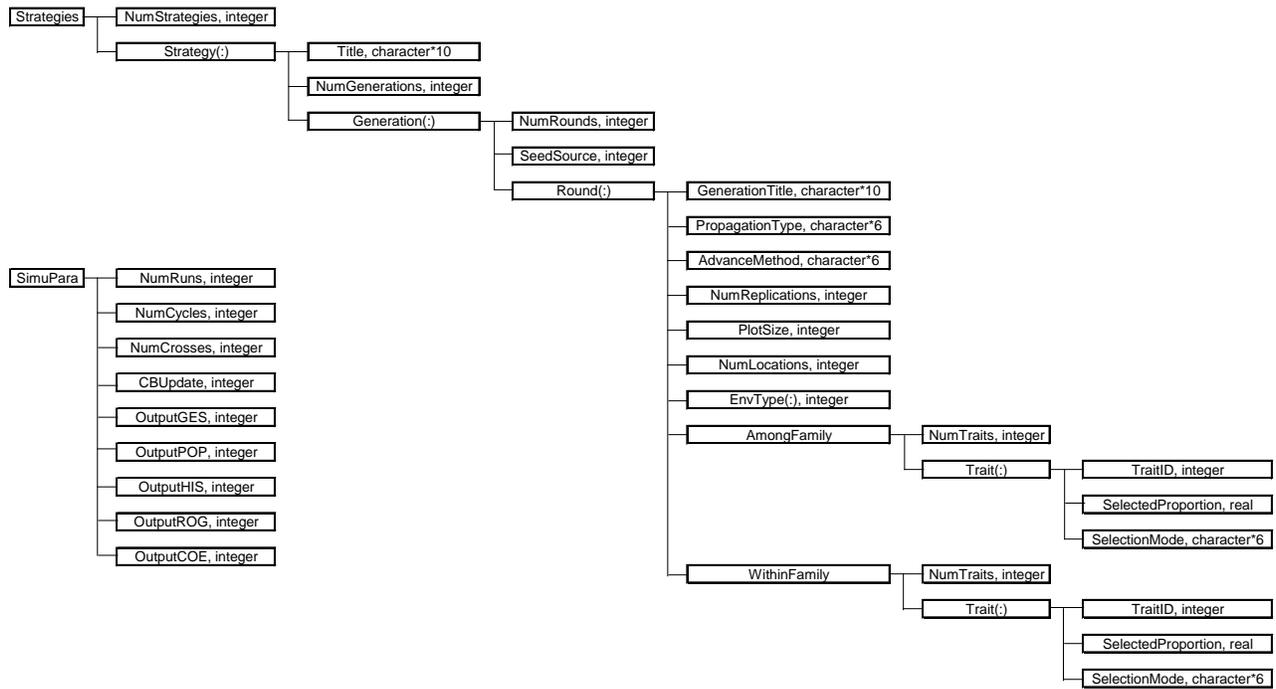


Fig. 1. Data structure of simulation parameters and breeding strategies.

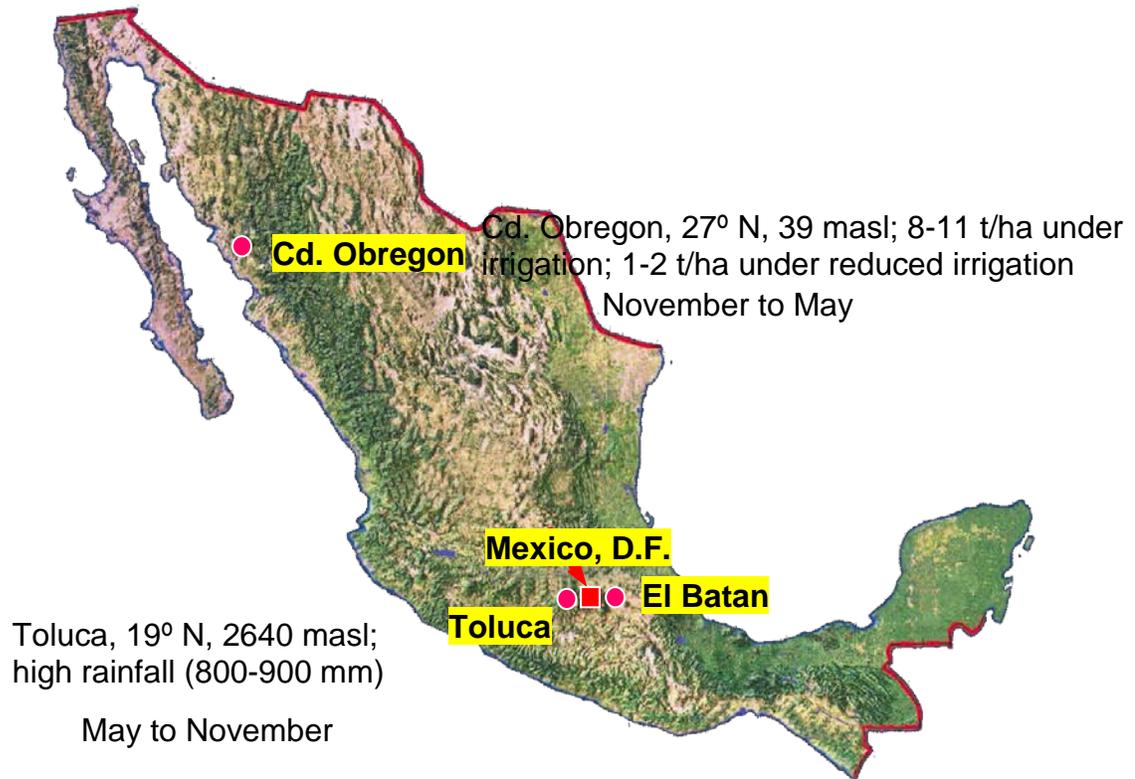


Fig. 2. The three Mexican selection sites used by CIMMYT's wheat breeding program.

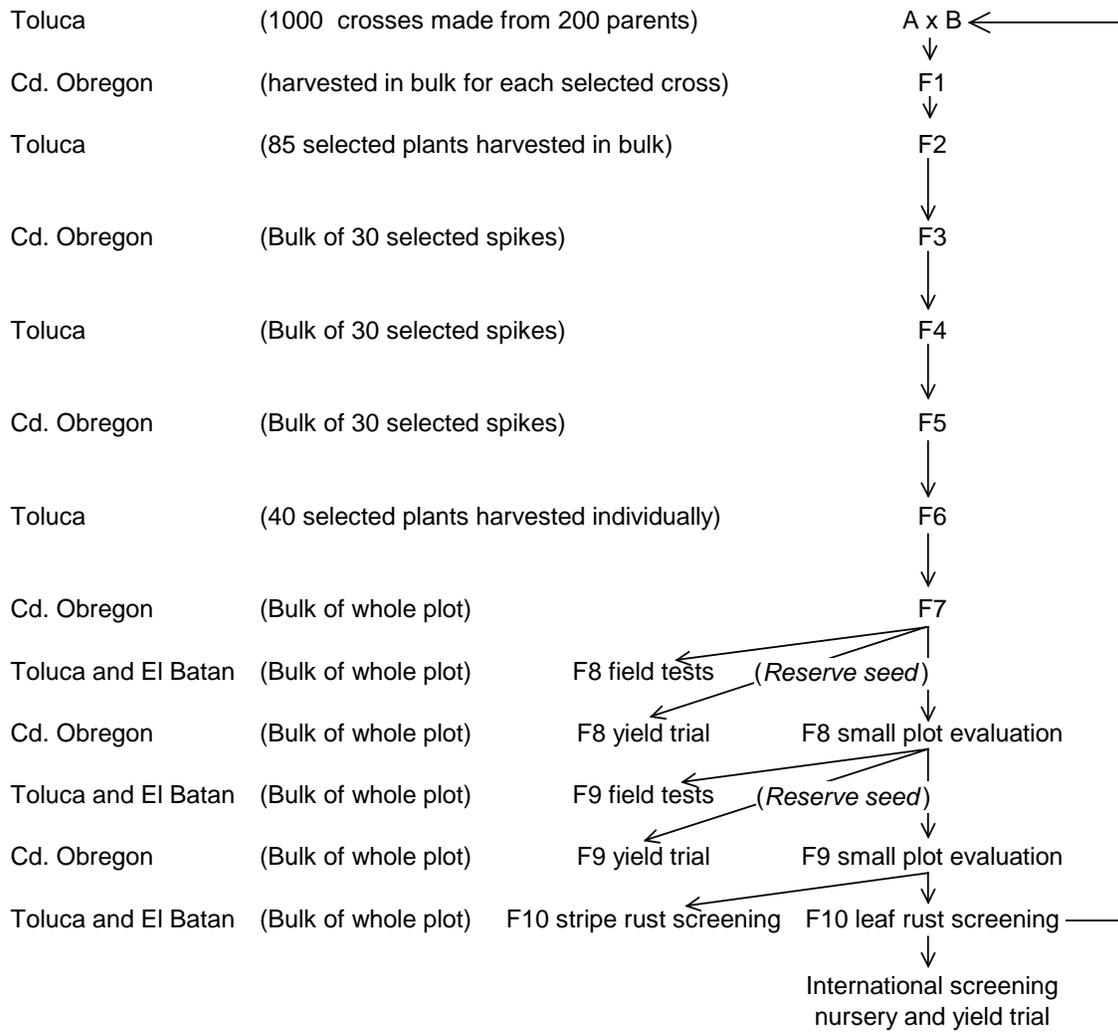


Fig. 3. Germplasm flow in the selected bulk selection method applied by CIMMYT's wheat breeding program, with sample population sizes.

Table 2. Definition of the modified pedigree and selected bulk selection methods in CIMMYT's wheat breeding program in QuLine.

```

!*****QMP file for QuCim 2.0*****
!Information for composite traits derived from simple traits in the GES file**
!Number of derived composite traits and numbers of simple traits in each
0

!No.      : the number for each composite trait, going as 101, 102, 103, etc.
!Type 1:  Addition of simple traits,
!        Y=a1*Y1+...+an*Yn (base index), ai is the weight
! 2:  Multiplication of simple traits, Y=a1Y1*...*anYn (multiplicative index)
! 3:  Selection index (optimum index), I=b'X, b=P^(-1)*G*a, P is phenotypic
!     covariances among traits, G is genetic covariances, calculated from
!     genotypic values. No markers can be included in this composite traits.
! 4:  Index for marker assisted selection (MAS), including all the markers and
!     one trait. I=bY*y+bM*m, y is the phenotypic value, m is the marker score
!     from regression analysis. bY=(VG-VM)/(VP-VM), bM=(VP-VG)/(VP-VM), VG is
!     the genetic variance, VM is the variance due to marker scores, VP is the
!     phenotypic variance. VG is calculated from genetic values.
! 5:  Index for marker assisted selection (MAS), including all the markers
!     and one trait. I=a1*y+a2*m, y is the phenotypic value, m is the marker
!     score from regression analysis.
!WTraits: which simple traits are used to define the composite trait.
!Weights: weight for each simple trait
!No.  Type  WTraits  Weights

!*****General information for the simulation experiment*****
!NumStr NumRun NumCyc NumCro CBUpdate OutGES OutPOP OutHIS OutROG OutCOE OutVar Cross  RMrimes PopSize
2      2      2      100  0      0      0      1      0      0      0      random  0      200

!*****Information for selection strategies to be simulated*****
!StrategyNumber StrategyName NumGenerations
2      SELBLK      10

!NR SS GT      PT      GA      RP PS      NL  ET...      Row 1
!      AT (ID SM SP)... Row 2
!      WT (ID SM SP)... Row 3
1 0 CB      self      bulk      1 10      1  2
!      0
!      0
1 0 F1      singlecross  bulk      1 20      1  1
!      7  2 B 0.98  3 B 0.99  4 B 0.85  6 M 0.99  8 T 0.90  7 M 0.98  9 T 0.97
!      0
1 0 F2      self      bulk      1 1000    1  2
!      7  2 B 0.99  3 B 0.99  5 B 0.90  6 M 0.99  8 T 0.99  7 M 0.99  9 T 0.99
!      7  2 B 0.95  3 B 0.99  5 B 0.40  6 M 0.85  8 T 0.60  7 M 0.90  9 T 0.50
1 0 F3      self      bulk      1 500     1  1
!      3  2 B 0.99  4 B 0.90  8 T 0.95
!      7  2 B 0.90  4 B 0.70  6 M 0.90  8 T 0.80  7 M 0.90  9 T 0.25 10 T 0.60
1 0 F4      self      bulk      1 625     1  2
!      4  2 B 0.99  5 B 0.96  6 M 1.00  8 T 0.95
!      7  2 B 0.90  5 B 0.65  6 M 0.95  8 T 0.80  7 M 0.90  9 T 0.20 10 T 0.60
1 0 F5      self      bulk      1 625     1  1
!      3  2 B 0.99  4 B 0.96  8 T 0.95
!      7  2 B 0.90  4 B 0.70  6 M 0.90  8 T 0.80  7 M 0.90  9 T 0.20 10 T 0.60
1 0 F6      self      pedigree  1 750     1  2
!      3  2 B 0.99  5 B 0.96  8 T 0.95
!      6  2 B 0.90  5 B 0.70  6 T 0.90  8 T 0.95  7 M 0.98  9 T 0.10
4 0 F7      self      bulk      1 70      1  1
!      7  2 B 0.85  4 B 0.70  6 M 0.98  8 T 0.85  7 M 0.96  9 T 0.70 10 T 0.75
!      0
!      6  2 B 0.97  5 B 0.70  6 M 0.99  8 T 0.98  7 M 0.99  9 T 0.90
!      0
!      1  3
!      1  4 B 0.90
!      0
!      1  1
!      1  1 T 0.40
!      0
4 0 PC      self      bulk      1 30      1  1
!      0
!      0
!      0
!      1  2
!      4  2 B 0.97  5 B 0.95  8 T 0.99  9 T 0.99
!      0
!      1  3
!      1  4 B 0.95
!      0
!      1  1
!      1  1 T 0.40
!      0
2 0 EPC      self      bulk      1 30      1  1
!      0

```

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```

                                0
CISNYT(T) self      bulk      1 30  1  2
                                1  5 B 0.98
                                0
1 0 CISNYT(B) self  bulk      1 30  1  3
                                1  4 B 0.98
                                0

```

!***** Information for selection strategies to be simulated *****

!Common parameters for all strategies in the QMP file (all values in one row).

```

! NumStr      : Any positive integer for the number of breeding strategies.
!              All these NumStr strategies need to be defined in this file.
! NumRun      : Any positive integer for the number of runs.
! NumCyc      : 0 or any positive integer for the number of breeding cycles.
! NumCro      : Any positive integer for the number of crosses to be made. Same
!              number of crosses will be made for all strategies in any cycle,
!              and same crosses will be made for all strategies in the 1st cycle.
! CBUupdate   : 0 (for no) or 1 (for yes), the indicator for updating the crossing
!              block after each breeding cycle.
! OutputGES   : 0 (for no) or 1 (for yes), the indicator for outputting the detailed
!              GE system information (all random effects will be assigned values).
! OutputPOP   : 0 (for no) or 1 (for yes), the indicator for outputting the detailed
!              population information (allele combination, genotypes, phenotypes etc).
! OutputHIS   : 0 (for no) or 1 (for yes), the indicator for outputting selection
!              history for each line in the final selected population.
! OutputROG   : 0 (for no) or 1 (for yes), the indicator for outputting cross
!              information (Rogers distance, lines retained, lines above parents etc).
! OutputCOE   : 0 (for no) or 1 (for yes), the indicator for outputting correlation
!              information (by traits and by environments).
! OutputVAR   : 0 (for no) or 1 (for yes), the indicator for outputting genetics
!              variances (by traits and by environments).
! Cross       : If random, QuCim will randomly select parents (including backcross
!              and topcross parent), otherwise QuCim will read the parents from
!              the specified external file. The file must exist.
! RMTimes     : Times of random mating with the final selected population after each cycle
!              0 means no random mating with the final selected population
! PopSize     : Number of individuals after random mating

```

!Information for each strategy

```

!Strategy number      : from 1 to NumStr.
!Strategy name        : maximum 30 characters.
!Number of generations : any integer. Crossing block is not included and viewed as
!                      generation 0 in QuCim.

```

!All generations have the same input format. The first two values for each generation are,

```

! NR : Number of selection rounds in the generation (1 for most generations).
! SS : Seed source indicator, 0 or 1.
!     0, seed is from round 1 for round 2 to NR-1.
!     1, seed is from round n-1 for round n (1<n<NR).
!     No effect for generations with one selection round. 0 and 1 have the same
!     effects for generation having 2 selection rounds.

```

!All selection rounds have the same input format (three rows for each).

```

! Row 1, GT : Generation title (maximum 10 characters).
!           PT : Seed propagation type, which can only be clone, self, random,
!                 backcross, topcross, DH, or noself. For generation 0, only clone
!                 and self are available. For generation 1, only random is available.
!                 However the crosses can be changed under QuCim windows application.
!           GA : Generation advance method, pedigree or bulk.
!           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 (0 or a valid environment type).
!                 All types except 0 should be defined in the GES file. An environment
!                 type will be assigned to the location with type 0 while simulating,
!                 depending on the occurrence frequency of each environment type in TPE.
! Row 2, AT : Number of among family selection traits, 0 (no more input required
!             for among family selection) or a positive integer. For each trait,
!           ID : Trait number, 0 (for marker score) or a valid trait number
!                 (defined in the GES file).
!           SM : Selection mode, T (top), B (bottom), M (middle), R (random), selected by proportions
!                 TV (top value), or BV (bottom value), selected by threshold values
!                 TN (top number), BN (bottom number), or RN (random) selected by specified numbers
!           SP : selected proportion (0.0 to 1.0), if SM=T, B, M, or R
!                 : truncated value (any real number), if SM=TV, or BV, i.e. &

```

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```
!           any family above SP (for SM=TV) or below SP (for SM=BV) will be selected
!           : number of families to be selected, if SM=TN, BN, or RN
! Row 3, WT : Number of within family selection traits, 0 (no more input required
!           for within family selection) or a positive integer. For each trait,
!           ID : Trait number, 0 (for marker score) or a valid trait number
!           (defined in the GES file).
!           SM : Selection mode, T (top), B (bottom), M (middle), R (random), selected by proportions
!           TV (top value), or BV (bottom value), selected by threshold values
!           TN (top number), BN (bottom number), or RN (random) selected by specified numbers
!           SP : selected proportion (0.0 to 1.0), if SM=T, B, M, or R
!           : truncated value (any real number), if SM=TV, or BV, i.e. &
!           any individuals above SP (for SM=TV) or below SP (for SM=BV) will be selected
!           : number of families to be selected, if SM=TN, BN, or RN
!Note: the among family selection for generation 0 is used define the female master.
!       the within family selection for generation 0 is used define the male master.
```

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Table 3. Definition of some common breeding methods in QuLine.

```

!*****General information for the simulation experiment*****
!NumStr NumRun NumCyc NumCro CBUupdate OutGES OutPOP OutHIS OutROG OutCOE OutVar Cross RMTimes PopSize
8      2      2      200    0      0      0      0      0      0      0      random  0      200

!StrategyNumber StrategyName NumGenerations
1              mass           1

!NR SS GT      PT      GA      RP PS  NL  ET...      Row 1
!              (ID SM SP)... Row 2
!              WT (ID SM SP)... Row 3
1  0  CB      self    bulk    1  1    1  1
0
0
1  0  S0      Singlecross pedigree 1  1    1  1
1  1  0.1 T
0

!StrategyNumber StrategyName NumGenerations
2              familyS1      1

!NR SS GT      PT      GA      RP PS  NL  ET...      Row 1
!              (ID SM SP)... Row 2
!              WT (ID SM SP)... Row 3
1  0  CB      Singlecross bulk    1  1    1  1
0
0
2  0  S0      random    pedigree 1  1    1  1
0
0
      S1      self    bulk    2  30    1  1
1  1  0.1 T
0

!StrategyNumber StrategyName NumGenerations
3              familyS2      1

!NR SS GT      PT      GA      RP PS  NL  ET...      Row 1
!              (ID SM SP)... Row 2
!              WT (ID SM SP)... Row 3
1  0  CB      self    bulk    1  1    1  1
0
0
3  0  S0      Singlecross pedigree 1  1    1  1
0
0
      S1      self    bulk    2  30    1  1
1  1  0.333 T
0
      S2      self    bulk    2  30    1  1
1  1  0.333 T
0

!StrategyNumber StrategyName NumGenerations
4              PedigreeSystem 8

!NR SS GT      PT      GA      RP PS  NL  ET...      Row 1
!              (ID SM SP)... Row 2
!              WT (ID SM SP)... Row 3
1  0  CB      self    bulk    1  1    1  1
0
0
1  0  F1      Singlecross bulk    1  10    1  1
0
0
1  0  F2      self    pedigree 1  1000  1  1
2  2 0.5 T  3 0.6 B
4  1 0.1 R  5 0.8 M  2 0.5 R  3 0.5 R
1  0  F3      self    pedigree 1  50    1  1
1  3 0.4 B
2  1 0.1 R  5 0.6 M
1  0  F4      self    pedigree 1  50    1  1
2  5 0.5 T  4 0.2 B
    
```

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```

1 0 F5      self    pedigree  1  30      1  4 0.1 R
1 0 F6      self    pedigree  1  30      1  1
1 0 F7      self    bulk       1  10      2  1 0.2 R    5 0.5 M
1 0 F8      self    bulk       3  10      1  1
1 0 F8      self    bulk       3  10      2  2 0.2 T    3 0.35 B    5 0.8 M    2 0.5 R
1 0 F8      self    bulk       3  10      1  1
1 0 F8      self    bulk       3  10      4  1 2 0 1
1 0 F8      self    bulk       3  10      2  2 0.2 T    3 0.2 B
1 0 F8      self    bulk       3  10      0

```

```

!StrategyNumber StrategyName      NumGenerations
5              ModifiedPedigree  8

```

```

!NR SS GT      PT      GA      RP PS    NL  ET...      Row 1
!              PT      GA      RP PS    AT (ID SM SP)... Row 2
!              PT      GA      RP PS    WT (ID SM SP)... Row 3
1 0 CB        self    bulk     1  1      1  1
1 0 F1        Singlecross bulk     1  10     1  1
1 0 F2        self    pedigree 1  1000   1  1
1 0 F3        self    bulk     1  50     2  2 0.8 T    3 0.8 B
1 0 F4        self    bulk     1  50     4  1 0.1 R    5 0.8 M    2 0.5 R    3 0.5 R
1 0 F5        self    bulk     1  50     1  1
1 0 F6        self    bulk     1  50     2  1 0.1 R    5 0.6 M
1 0 F7        self    bulk     1  50     2  3 0.5 T    1 0.2 B
1 0 F8        self    bulk     1  30     1  4 0.1 R
1 0 F8        self    bulk     1  30     1  1
1 0 F8        self    bulk     1  30     2  1 0.2 R    5 0.5 M
1 0 F8        self    pedigree 1  30     1  1
1 0 F8        self    bulk     1  10     2  2 0.8 T    3 0.8 B
1 0 F8        self    bulk     1  10     3  1 0.5 R    5 0.8 M    2 0.9 R
1 0 F8        self    bulk     3  10     1  1
1 0 F8        self    bulk     3  10     4  1 2 0 1
1 0 F8        self    bulk     3  10     2  2 0.2 T    3 0.2 B
1 0 F8        self    bulk     3  10     0

```

```

!StrategyNumber StrategyName      NumGenerations
6              SelectedBulk    8

```

```

!NR SS GT      PT      GA      RP PS    NL  ET...      Row 1
!              PT      GA      RP PS    AT (ID SM SP)... Row 2
!              PT      GA      RP PS    WT (ID SM SP)... Row 3
1 0 CB        self    bulk     1  1      1  1
1 0 F1        Singlecross bulk     1  10     1  1
1 0 F2        self    bulk     1  1000   1  1
1 0 F3        self    bulk     1  50     2  2 0.8 T    3 0.8 B
1 0 F4        self    bulk     1  50     4  1 0.1 R    5 0.8 M    2 0.5 R    3 0.5 R
1 0 F5        self    bulk     1  50     1  1
1 0 F6        self    bulk     1  50     2  1 0.1 R    5 0.6 M
1 0 F7        self    bulk     1  50     2  5 0.5 T    2 0.9 B
1 0 F8        self    bulk     1  30     1  4 0.1 R
1 0 F8        self    bulk     1  30     1  1
1 0 F8        self    bulk     1  30     2  1 0.2 R    5 0.5 M
1 0 F8        self    pedigree 1  30     1  1
1 0 F8        self    bulk     1  10     2  2 0.8 T    3 0.8 B

```

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```

1 0 F7      self      bulk      1 10      3 1 0.6 R    5 0.8 M    2 0.9 R
1 0 F8      self      bulk      5 20      4 1 2 0 1    2 2 0.2 T    3 0.2 B
0
0
!StrategyNumber StrategyName NumGenerations
7              DHF2          3

!NR SS GT      PT      GA      RP PS      NL ET...      Row 1
!              AT (ID SM SP)... Row 2
!              WT (ID SM SP)... Row 3
1 0 CB        self      bulk      1 1      1 1
0
0
1 0 F1        Singlecross bulk      1 10      1 1
0
0
1 0 F2        self      pedigree 1 1000    1 1
2 2 0.8 T    3 0.8 B
4 1 0.1 R    5 0.8 M    2 0.5 R    3 0.5 R
1 0 DHF2      DH        bulk      3 10      3 2 0 0
1 3 0.1 T
0

!StrategyNumber StrategyName NumGenerations
8              DHF3          3

!NR SS GT      PT      GA      RP PS      NL ET...      Row 1
!              AT (ID SM SP)... Row 2
!              WT (ID SM SP)... Row 3
1 0 CB        self      bulk      1 1      1 1
0
0
1 0 F1        Singlecross bulk      1 10      1 1
0
0
1 0 F2        self      pedigree 1 1000    1 1
2 2 0.8 T    3 0.8 B
4 1 0.1 R    5 0.8 M    2 0.5 R    3 0.5 R
1 0 F3        self      pedigree 1 50      1 1
2 2 0.8 T    3 0.8 B
1 3 0.1 R
1 0 DHF3      DH        bulk      4 10      3 2 0 0
1 3 0.1 T
0

```

4. Steps for running a simulation experiment using QU-GENE engine and QuLine

General information about the process for using QuLine was shown in Fig. 4.

4.1 Build the QUG file for the engine input

The QUG file, the input for the QU-GENE engine, contains all the information to define the GE system and populations. Table 1 can be used as a template to build a QUG file fitted to any breeding program. Normally, several QUG files need to be built for a simulation experiment. For example, if you want to investigate the effects of different genetic models (e.g., additive, additive and dominance, and additive and dominance and epistasis; other parameters are the same) on selection, three QUG files corresponding to these three kinds of genetic models need to be built. If you want to compare the effect of different numbers of genes (e.g., 10, 20, and 100 genes) on selection, three QUG files, one for each of these three gene numbers, need to be built.

4.2 Run the QU-GENE engine to generate GES and POP files

Double click the executable file “QUGENE.exe”, which will run the engine and show some interfaces. There are two ways to run a set of QUG files. You can put all the QUG file names into another pure text file with the extension WQG. If you choose option 1, the engine will list all the WQG files for your input. Then the engine will run all the QUG files listed in the WQG file. If you choose option 2, the engine will list all the QUG files for your input. Once the engine completes the QUG file you input, you may repeat the process to run another QUG file. You may also open a set of QUG files and run them one after another under this option.

There is a summary message after the engine executes all selected QUG files. The message “Completed Successfully” is shown on the screen once the engine has successfully read all the data in the QUG file and written the GES and POP output files, and the defined diagnostic information. The GES and POP files from this QUG file are then ready to be used by the application modules. If this message does not appear, errors may be present in the QUG file. You should go back to the QUG file and find out where the error is, correct it, and re-run the QUG file until you see the message “Completed Successfully.” In general, only one GES file will be generated; however, more than one POP file can be generated from a QUG file.

4.3 Build the QMP file for the simulation experiment

A graphical user interface is available at UQ to help you build the QMP file. However, you may also build the pure text file directly. If you wish to build it on your own, Tables 2 and 3 can be used as a template.

4.4 Build the MIO files

If you run the experiment in the computer cluster located at The University of Queensland, Brisbane, Australia, you don't need to build the MIO files. You just put all the GES files, POP files, and QMP files, along with the QuLine, in a specific folder. The cluster can help to build the MIO files for all possible GES by POP by QMP combinations, and then make QuLine run on all the combinations. If you run the experiment in other personal computers, you need to edit all the MIO files, which are also in pure text format. Please remember the default MIO file name used by QuLine is "QuLine.mio". When building the MIO file to run QuLine on personal computers or putting the three kinds of files together to run QuLine on the cluster, it is your responsibility to make sure that the three files in one MIO are compatible. For example, you cannot put a GES with 10 genes and a population defined from a GE system with 20 genes in the same MIO file. If there are 10 traits in the QMP file, then all 10 traits have to be defined in GES file. If the breeding materials are grown in three different environment types, all three environment types have to be defined in the GES file.

4.5 Run QuLine

The QuLine Windows application (for demonstration) can call up any MIO files, while the QuLine console application (for simulation experiments) will automatically call the MIO file named "QuCim.mio". Hence the file "QuCim.mio" must exist before running the QuLine console application. The three files (a GES, a POP, and a QMP) specified in the "QuCim.mio" must of course also exist and be accessible. Some results are recorded in the output files while QuLine is running. At the top of these output files, you will find which GES, POP and QMP were used.

4.6 Outputs from running QuLine

There are 12 output files from running QuLine that have the same prefix defined in the MIO file but different extensions.

(1) *Crosses retained after each round of selection (*.CRO)*

The number of crosses retained after each round of selection is recorded in this file. Information in this file can be used to compare the genetic diversity in the final selected population from each selection strategy.

(2) *Detailed GE system information (*.DEB)*

Detailed information about the GE system is recorded in this file, as required by the user. If there are random gene effects in the GE system, valid values will be assigned to those random effects. This file can be quite big when there is a large number of models.

(3) *Error message (*.ERR)*

The processing and error messages that come up while QuLine is running are recorded in this file. When QuLine exits abnormally, the messages in this file may help find the problem.

(4) *Mean genotypic value in the final selected population (*.FIT)*

Mean genotypic values (also called fitness) and the values adjusted by target genotypes in the final selected population for all traits defined in the GE system, and in all environment types and the TPE are recorded in this file. The genotypic value of an individual plant (denoted as F for fitness) in each environment type is calculated from the genetic effects defined in the GE system. The adjusted genotypic value is defined as $F_{ad} = \frac{F - TG_l}{TG_h - TG_l} \times 100$, where TG_l and TG_h are the genotypic values for the two extreme target genotypes with the lowest and the highest trait values in the GE system, respectively. This standardization is useful specifically when diverse GE systems are used to compare the performances of different breeding strategies. The information in this output file can be used to calculate the genetic gain from each selection strategy.

(5) *Genes fixed in the final selected population (*.FIX)*

This file contains the percentage of fixed genes for all traits and the percentage of fixed genes for each trait.

(6) *Gene frequency in the initial and selected populations (*.FRE)*

This file contains the change in gene frequency for each gene before and after selection.

(7) Hamming distance in the final selected population (.HAM)*

Hamming distance is a measure of the difference in allelic combinations present in any genotype compared to the highest target genotype in the GE system. A small Hamming distance means that a specific genotype is close to the highest target genotype. When there is no epistasis, a clear relationship occurs between Hamming distance and the adjusted genetic value. However, Hamming distance under epistasis needs to be carefully explained, as a small change in Hamming distance may result in a great change in genetic value when epistasis is present.

(8) Selection history of all selected lines in the final selected population (.HIS)*

The selection history of each line in the final selected population is recorded in this file. In the selection history of a line, "C" stands for cross and is followed by the cross number; "-" is used to distinguish each generation. "0" before a number means the bulk generation advance method was used. Otherwise, pedigree was used.

(9) Population information (.POU)*

The allele combination, genotypic value, and phenotypic value of each line in the initial and final selected populations are recorded in this file.

(10) Allocated resources for all simulated strategies (.RES)*

Number of families and number of individual plants in each generation for each simulated strategy are recorded in this file, and so are the trait and environment correlation coefficients.

(11) Rogers distance between the two parents of each cross (.ROG)*

The Rogers distance and the number of lines retained from each cross are recorded in this file. At the end of each breeding cycle, the number of lines above the middle parent and the number of lines above the better parent from each cross are also recorded.

(12) Genetic variance in the final selected population (.VAR)*

This file contains the genetic variance and heritability in the initial population and the selected population. When there is no epistasis, the genetic variance is decomposed into additive variance and dominance variance. Heritability in the narrow and the broad sense is given. If there is epistasis, no heritability is given, because the additive, dominance, and epistatic variances cannot be estimated by the population itself. The additive and non-additive genetic variances are estimated using North Carolina Design II (NCII).

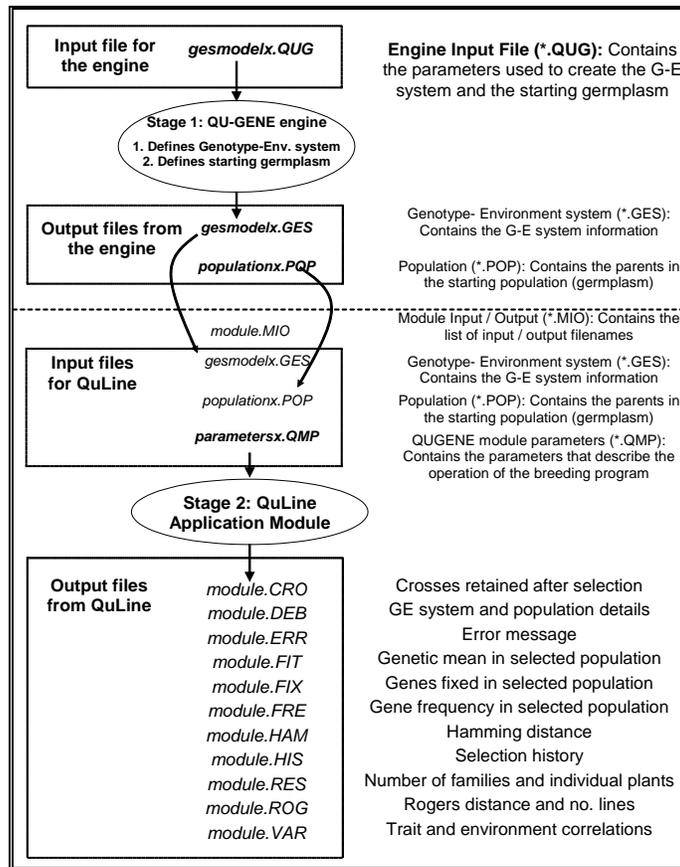


Fig. 4. Process for running the QU-GENE strategic application QuLine.

5. Peer-reviewed publications using QU-GENE and QuLine

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- Wang, J., X. Wan, H. Li, W. Pfeiffer, J. Crouch, J. Wan. 2007. Application of identified QTL-marker associations in rice quality improvement through a design breeding approach. **Theor. Appl. Genet.** 115:87–100
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- Wang, J., R. P. Singh, H.-J. Braun, and W. H. Pfeiffer. 2009. Investigating the efficiency of the single backcrossing breeding strategy through computer simulation. **Theor. Appl. Genet.** 118: 683-694.
- Wang, J., S. C. Chapman, D. G. Bonnett, and G. J. Rebetzke. 2009. Simultaneous selection of major and minor genes: use of QTL to increase selection efficiency of coleoptile length of wheat (*Triticum aestivum* L.). **Theor. Appl. Genet.** (in press).
- Ye, G., D. Moody, L. Emebiri, and M. van Ginkel. 2007. Designing an optimal marker-based pedigree selection strategy for parent building in barley in the presence of repulsion linkage, using computer simulation. **Aust. J. Agric. Res.** 58: 243–251.

6. Abbreviations and acronyms

B, CIMMYT's wheat breeding location at El Batan, Mexico.

CAAS, Chinese Academy of Agricultural Sciences, Beijing, China.

CGIAR, Consultative Group on International Agricultural Research.

CIMMYT, International Maize and Wheat Improvement Center, headquartered in Mexico.

CRO, extension of a QuLine output file that contains the number of crosses retained after each round of selection for each model, run, strategy, and cycle.

DEB, extension of a QuLine output file that contains detailed information about the GE system for each model and about the population for each model, run, strategy, and cycle. This output file is normally used for debugging; hence the name.

DH, doubled haploid.

F, filial generation; F₀ is used for the crossing block.

FIT, extension of a QuLine output file that contains the average genotypic value in the final selected population for each defined trait and in each defined environment type and TPE.

FIX, extension of a QuLine output file that contains the percentage of genes fixed in the final selected population for all defined traits in each defined environment type and TPE.

FRE, extension of a QuLine output file that contains the gene frequency in the final selected population for each defined trait and in each defined environment type.

GCP, Generation Challenge Programme of CGIAR

GE, genotype and environment, or genotype by environment.

GES, extension of the QUGENE output files containing the necessary information about the genotype and environment systems. One QUG file will generate one GES file.

GRDC, Grains Research and Development Corporation, Australia.

GRN/SPK, grains per spike.

HAM, extension of a QuLine output file that contains the Hamming distance in the final selected population for each defined trait and in each defined environment type and TPE.

HarvestPlus, the Biofortification Challenge Program of CGIAR.

HIS, extension of a QuLine output file that contains the selection history of each line in the final selected population.

LR, leaf rust.

MAS, marker-assisted selection.

MIO, extension of files that QuLine will use to identify the GES, POP and QMP file names.

POP, extension of the QUGENE output files containing the necessary information about populations. One or more POP files can be generated from one QUG file.

QMP, extension of the QuLine input files containing information necessary for a simulation experiment.

QuCim, the original version of QuLine.

QuLine, a QU-GENE application breeding simulation module that simulates breeding programs of self-pollinated crops.

QUG, extension of QUGENE input files.

QU-GENE, a simulation platform for quantitative analysis of genetic models.

QUGENE, the QU-GENE engine, used to generate the genotype and environment systems and populations.

RES, extension of a QuLine output file that contains the number of families and the number of individual plants in each generation for each simulated strategy.

ROG, extension of a QuLine output file that contains the Rogers distance for each cross and the number of lines retained after each round of selection. At the end of a breeding cycle, the number of lines above the mid-parent and the number of lines above the better parent are also given in this file.

SP, small plot evaluation in the field.

T, CIMMYT's wheat breeding location in Toluca, Mexico.

TKW, thousand kernel weight.

TPE, target population of environments for a breeding program.

UQ, The University of Queensland, Brisbane, Australia.

VAR, extension of a QuLine output file that contains the genotypic variances and heritabilities in the initial population and populations after selection.

WQG, extension of the input file for the QU-GENE engine. This file contains the names of a number of QUG files. The engine will run all the QUG files defined in this file.

YR, yellow rust.

YT, yield trial.

7. Acknowledgments

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