Chapter 2
Genetic Analysis Methods of Quantitative Traits in Wheat

Abstract It is very important of understanding how to construct the genetic population and genetic maps, what methods should be used, etc., to researchers; so in this chapter, the types and quality of genetic population, construction methods of genetic population, types of genetic markers, and statistical methods of QTL mapping were introduced; moreover some new methods and key notes from our study experience were especially provided.

Keywords Genetic populations · Genetic markers · Population construction · Populations Quality · QTL mapping · Genetic analysis methods

In order to conduct genetic analyses of wheat quantitative traits, it is essential to establish proper genetic populations, select appropriate DNA markers, construct genetic maps and conduct QTL mappings. As the old saying stated, "traditional breeding often depends on experience, while molecular breeding relies on materials." Here the word "materials" simply refers to the genetic populations for quantitative trait studies. Therefore, this chapter presents the type and quality of genetic populations followed by a brief introduction of the genetic analyses of wheat quantitative traits.

2.1 The Types and Quality of Genetic Populations

2.1.1 The Types of Genetic Populations

A genetic population, in a narrow sense, is often derived from a F_1 hybrid resulted from a cross between two pure breeding parents with distinct alleles, and it represents all genotypes of the family. In theory, such population should cover all loci, including those of homozygous and those of heterozygous. The basic guideline for creating an ideal genetic population is to avoid any man-made interruptions or selections during the population construction. However, to gain all genotypes is impossible due to infertility derived from some reproductive disorders, and mortalities or loss because of various environmental stresses or human factors.

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Currently, two types of genetic populations for genetic map construction are used: temporary population and permanent population. The former covers F_2 and its derivatives of F_3, F_4 and BC (backcross) lines, and the latter includes doubled haploid (DH), recombinant inbred line (RIL), immortalized F_2 (IF_2), and near-isogenic lines (NIL) populations. In recent years, association mapping based on (LD) linkage disequilibrium has been widely used to select natural genetic populations containing various variations, and such population can be a commercial variety, a newly bred variety candidate, or germplasm. This suggests that these natural populations belong to permanent populations.

Based on the accuracy of QTL mapping, there are two types of mapping populations: primary population and secondary population (also known as fine mapping population). The former includes F_2 and its derivatives (e.g., F_3, F_4 lines), BC_1, BC_2F_X, DH, RIL, and IF_2 (derived from DH or RIL). Due to the interference of the genetic background, the confidence intervals of the QTLs identified with these populations are often larger than 10 cm. According to the population sources, the secondary mapping populations can be further divided into two groups: those derived from primary mapping populations via further selections [(e.g., NILs, residual heterozygous lines (RHLs), and QTL isogenic recombinants lines (QIRs)]; those substitution populations that have no relationship with the primary populations, [e.g., introgressive lines (ILs), single-segment lines (SSLs), and chromosome segment substitution lines (CSSLs)]. The QTL fine mapping population can eliminate the interferences of various genetic backgrounds, thereby gain better accuracy in QTL mapping.

The following is a brief introduction of the characteristics of several commonly used mapping genetic populations and a few key points to consider during the constructions of QTL mapping populations.

2.1.1.1 F_2 and Its Derived F_3 Population

In theory, the F_2 population should represent all the possible recombinants due to the random fusions of male and female gametes produced by selfing F_1 individuals and thus should generate abundant genetic information. This unique population can be easily created as it is rather simple. However, using F_2 as mapping populations has several significant limitations, including the following: (1) since phenotypic identifications are based on the individual plants, detection of many QTL traits with low heritability can be difficult; (2) F_2 is a temporary unstable population and long-term maintenance is an issue as its genetic structure will change, leading to difficulties to conduct multiple experiments each year at multiple locations after its sexual reproduction; (3) the presence of heterozygous genotypes in F_2 makes identification of homozygous or heterozygous dominant loci difficult, leading to low mapping accuracy. Therefore, only QTLs with significant effects and stable expressions can be detected when F_2 population is used. An alternative to this issue is the use of "mixed F_3" families derived from F_2. This approach involves the use of mixed DNA of all F_3 individuals to analyze their F_2 individual genotypes. A genetic

map can also be constructed when each F_3 individual is evaluated. For each locus, segregation ratio will be 3:2:3, instead of 1:2:1. This is because, for a F_2 heterozygous locus, there is only one chance, but two in F_3. The disadvantages, however, are labor intensive and the increase in sampling errors.

2.1.1.2 Backcross Population

BC_1 is the result of F_1 being backcrossed with one of its two parents and has been commonly used as mapping population. Since each segregating locus in BC_1 has only two genotypes, the resulted population represents the segregation ratio of F_1 gametes. Compared to the F_2 population, BC_1 provides the highest mapping efficiency. Yet, similar to F_2, the BC_1 population can be used for only one generation, cannot be maintained for a long term, and can provide limited information. For these reasons, direct use of BC_1 for QTL mapping is rare, unless there is a need for some special studies (e.g., cross-incompatibility). A permanent BC_1F_X population can be created via the repeated selfings of each individual line in BC_1.

2.1.1.3 DH Population

Doubled haploid (DH) lines are normally created through in vitro cultured anthers/immature microspores or ovaries/ovules (representing male or female gametes, respectively) followed by genome doublings (Fig. 2.1). Therefore, each DH

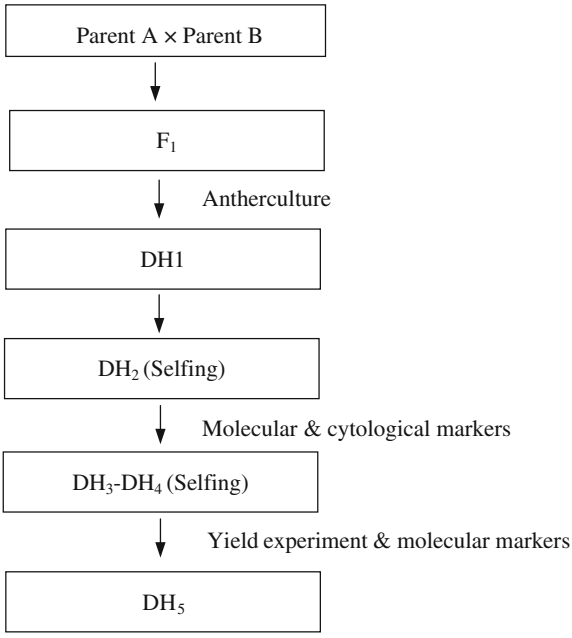

Fig. 2.1 Schematic diagram of DH generation and evaluation

line is completely homozygous and is a "permanent population." The advantages of such populations include the following: (1) they can be used for multiple experiments to minimize the experimental errors and (2) can be planted under various environments and in multiple years to study interactions between genotype and environment as DH lines are the most ideal materials. Moreover, the DH lines are also ideal for QTL fine mapping (Li et al. 1996) because their genetic structures represent the segregations and recombinations of alleles during F_1 gamete formation. The disadvantages of DH lines are as follows: (1) generation of DH lines often depends on the anther culture technology, resulting in interruptions of genetic structures and biased partitions due to possible differential responses of different gamete genotypes to the culture conditions; (2) limited rate of recombinations and lack of heterozygosities because there is only one meiotic division during gamete formation; and (3) DH lines can be used only for analyses of QTL additive effect, but not for dominant effect, which would affect the QTL mapping accuracy (Li et al. 2005).

2.1.1.4 RIL Population

A RIL is a permanent population and is developed by single-seed descent (select one individual plant each generation) of F_1, and this selfing process continues for multiple generations. Each individual genotype within the RIL population is a homozygous stable line, while different individuals may represent various genotypes. Similar to the DH populations, the RILs are permanent and can be experimented multiple times at various sites each year. However, compared with DH lines, RILs are unique that rates of recombinants within each linkage interval of the two homologous chromosomes increase significantly due to the repeated selfing process. Therefore, the use of RILs can break different QTLs located in the same chromosomal region, indicating that the RILs are ideal materials for QTL mapping (Burr 1988; Lin et al. 2008). The disadvantage of RIL population is that developing RILs are time-consuming and some lines may be eliminated during their continuous selfing process, leading to biased segregation.

2.1.1.5 NIL Population

Derived from a F_1 that was backcrossed repeatedly to one of its parents, the NIL population consists of lines with the same or similar genetic backgrounds, and yet with the variations only present in a few chromosomal regions. Hence, the NIL population is unique because the numbers of molecular markers required for mapping the targeted genes are fewer than other populations. Under the same genetic background, the NIL actually allow multiple QTLs that affect the same trait to break into individualized Mendel factors, convert the quantitative traits to qualitative traits, and thereby eliminate the interferences derived from genetic backgrounds and remove the masking effects of major QTLs over minor QTLs. NIL can be used to conduct map-based cloning of those targeted genes and fine gene mapping (Yano et al. 1997).

2.1.1.6 Segment Introgression Lines

A segment introgression line (also known as chromosome segment substitution line) is developed by crossing the F_1 with one of its parents repeatedly, by which a chromosome fragment of a variety is introgressed into the chromosome of the other. The desirable backcrossing generations can vary and often are based on the aims of the particular breeding and research programs. For breeding purpose, backcrossing two to three times is good enough when the transferred donor's DNA is about 12.5–6.25 % of the recipient's DNA. On the other hand, the backcrossing times should be increased so that a population with various DNA fragments can be created for QTL analyses.

2.1.1.7 Immortalized F_2 Population

Immortalized F_2 population (IF_2) was initially proposed by Hua et al. in (2003) and is unique as it combines the advantages of the segregating F_2 and the eternal RIL populations. The IF_2 is developed by the designed "2–2 crosses" between the homozygous lines of the eternal population. Importantly, the IF_2 population can not only provide rich information similar to the F_2 population so that the effects of both dominant and epistatic can be estimated effectively, but also, acted as the RIL or DH lines, can produce enough seeds to meet the demand for multiple trials at various sites each year, leading to gaining accurate phenotype data and identifying the closely linked QTL markers effectively.

Additionally, the IF_2 population can be used for heterosis QTL mappings since it can be trialed repeatedly at multiple sites within multiple years, which is impossible if F_2 or RIL eternal population is used alone. The main drawbacks to use IF_2 are (1) making massive cross combinations are both labor intensive and challenging, not all crosses can give rise to enough seeds, resulting in insufficient data and (2) completely random crosses are difficult to implement because heading time of RIL or DH populations can be different. These factors can be an issue as the established eternal IF_2 population may make the predicted data to be biased, and consequently lead to an abnormal population structure that has biased QTL locations and incorrect effect evaluations.

2.1.1.8 Fine Mapping Populations

Fine mapping populations include NILs, RHLs, QIRs, ILs (DNA segment introgression lines), single-segment substitution lines (SSSLs), and CSSLs.

The populations of NILs and ILs have been presented and shown below are discussions on the other remaining four mapping populations.

Residues of Alloplasmic Lines

Residues of alloplasmic lines (RHLs) are populations that consist of individuals with the presence of one or a few traits contributed by one parent, while other characters at other loci are derived from the other parent. Segregations of traits at the loci being examined occur constantly. RHLs have similar genetic background and can be used for marker-assisted selection, but cannot be used for epistatic effect evaluation.

QTL Isogenic Lines

QIRs are developed by a preliminary mapping initially using a small population followed by fine mapping using a large population. Each individual within the large population will have one recombination event occurring at the related QTL locus with no change at any remaining loci. QIR has both advantages (e.g., it is easy to construct and the molecular marker distance can be shorter than 1 cm) and drawbacks (e.g., background interference is present, and there are epistasis effect cannot be identified).

Single-Segment Substitution Lines

SSSL is very similar to NIL and is also developed through multiple generations of backcrossing (Fig. 2.2). An ideal SSSL should maintain all recipient genetic background with the exception of the targeted QTL DNA segment that comes from the donor's chromosome, strongly suggesting that SSSL can be used for fine mapping of single QTL. However, during the backcross process, the QTL gained via the preliminary mapping should be used for assistance selection, resulting in issues such as labor intensive and tedious.

Chromosome Segment Substitution Line

Different from SSSL, CSSLs are a series of NILs in which the substituted segments of the wide population contain the entire information of the donor, while each CSSL carries one or more donor chromosome segments in the genetic background of the recipient. The main characteristic of CSSLs is that the substituted segments of each CSSL are stable. As a result, CSSLs are useful for genetic studies in terms of the detection and fine mapping of QTLs for genome-wide target traits, and for studying the interactions between QTLs.

The introgression of fragments is mainly achieved through genetic recombination. Through backcross breeding, the lines carrying any genomic region can be produced. The selection mode adopted in the backcross process can be various; the final target should be that the lines have a single homozygous chromosome with the donor, while the other segments of the chromosome are from the acceptor parent.

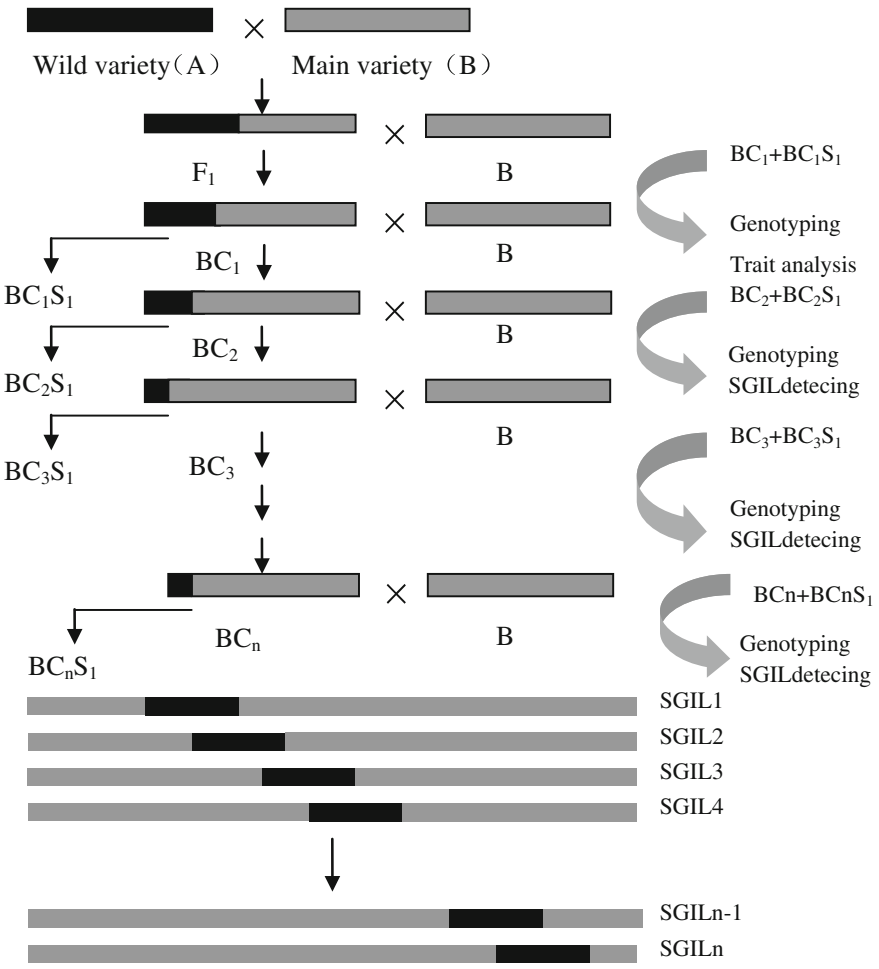

Fig. 2.2 Schematic diagram of SSSL construction (Reprinted from Zhang et al. 2004)

2.1.2 Genetic Population Construction and Some Key Notes to Consider

2.1.2.1 Construction Method

It is true that constructions of genetic populations should be based on each specific population even though most of which need to generate their F_1 as the initial step and share some common points.

F_2 Population Construction

A F_2 population is produced by selfing F_1 individuals. The key points include the following: their F_1 initial parents should show significant polymorphism the number of hybrid ears should be determined based on the F_2 population size, and each individual plant derived from each F_1 seed is a representative of the F_2 population.

IF_2 Population Construction

To establish an IF_2 population, first divide the DH or RIL population into two groups, each of which consists of a certain number of lines. Second, select one line from each group to make a cross match, followed by doing the same repeatedly from using the remaining lines of each group. After the 1st round crosses, a population with 1/2 of the DH or RIL lines can be obtained, and the 2nd round crosses will result in the population size equal to that of DH or RIL lines, thereby a whole set of IF_2 is created. This progress can be repeated yearly or one can make enough seeds per year to conduct trials at multiple sites per year.

BC_1 Population Construction

To create a BC_1 population, the F_1 is crossed back with one of its initial two parents. The backcross numbers are within 10–100 ears to develop a large population for the following desired studies.

NIL Population Construction

NIL is generated by crossing BC_1 with the same parent for at least four more generations (BC_5), resulting in the genetic compositions of the two isogenic lines are almost identical, with the exception of the targeted traits. The individuals selected for the backcrosses during the NIL developing process should be determined based on the NIL's targeted traits (e.g., ear size, plant height, grain weight, disease resistance, and quality). In addition to the field observation on the target traits, trait identification should be conducted by combining the biochemical markers with the DNA markers to speed up the NIL construction process (Fig. 2.3). Developing a NIL with 3–4 targeted traits using the same population is considered to be cost-effective.

RIL Population Construction

RIL populations are established via single-seed selections from individual plants of a F_2 population and the detailed process includes creating a F_2 population by selfing F_1, selecting 300 or more individuals (randomly select those showing significant

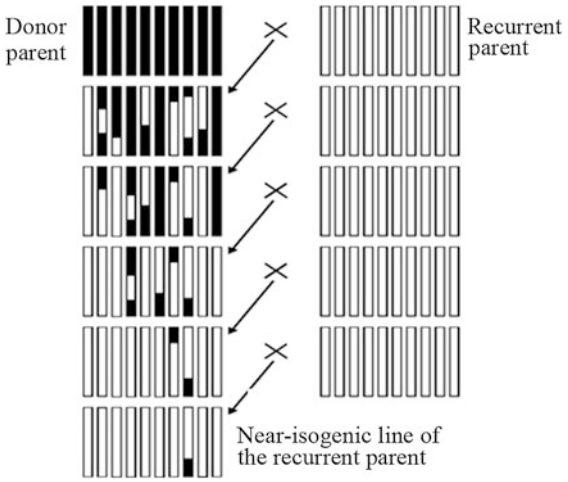

Fig. 2.3 Schematic diagram of NIL construction (Reprinted from Fang et al. 2001)

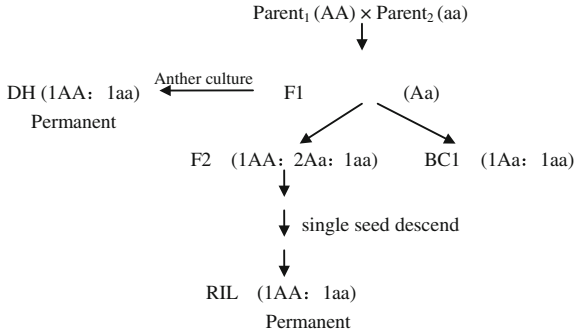

Fig. 2.4 Schematic diagram of genetic population constructions

polymorphisms) from the segregating F_2 population, and labeling them clearly and selfing the selected F_2 plants to obtain the F_3 seeds; this process is repeated until at least F_6 generations when all traits of each F_6 line are stabilized, which can be used for QTL mappings (Fig. 2.4). Noticeably, RIL construction is based on the "single-seed descent" principal, yet some genotypes can be eliminated due to poor sowing conditions if only one seed of each line is sown per generation, resulting in the number of lines in the population does not meet the QTL mapping requirements. To solve this issue, we select one ear/line/generation and sow all seeds in a single row starting from the F_2 generation until the F_6 generation when all traits are stabilized and are ready for QTL mapping.

DH Population Construction

DH populations are normally derived from the in vitro-cultured gametophytic cells of F_1 hybrids (known as anther/ovule culture) followed by genome doubling,

although haploids can also generated via cultured zygotes of some rare interspecific hybrids by eliminating the whole genome of one parent. Recently, a novel approach to inducing haploids is developed: when a centromere mutant (cenh3) is crossed to a wild type, the mutant genome is eliminated, and hence DH lines of the paternal or maternal wild parent are generated after genome doubling. In our laboratory, we develop wheat DH lines through anther culture. One of the key factors for successful DH production is to culture the anthers containing embryogenic microspores at mid-uninucleate to late uninucleate stages. Specifically, F_1 hybrid's anthers are harvested, in the field prior to heading, when the spike length reaches 2/3 of leaf sheath in the northern winter wheat region, and young panicle length is at 1 cm of the auricles or spikes and auricles are equal in length in the south of Huanghuai district, respectively. Importantly, anther length is about 2/3 of its total length and anther should look green and opaque.

Prior to culturing, ear with the sheath (after other leaves are removed) is disinfected in 70 % alcohol for 10 s after which sheath is removed. Anthers are taken out from the outer and inner glumes and placed onto the culture medium for cell de-differentiation or callus induction. The whole process is conduced under strict sterile condition in a laminar flow hood. The initial culture should be in the darkness and the tissue culture room temperature should maintain at 28–30 °C.

Cultures are transferred onto the plantlet initiation medium when callus size is about 1–1.5 mm in diameter, which requires about 30 days de-differentiation. This process takes about 7–15 days at conditions of 23–25 °C and daylight of 10 h. Plantlets of 2–3 cm long are then transferred onto a new medium for their further development for 7 days after which being placed in a growth chamber with temperature of 6–10 °C and light until mid-October. Plantlets are finally taken out and planted in a plot where transparent plastic film is provided on the top for acclimatization. Ten days later, the plastic cover can be removed and plants should be ready to go through the winter.

To double the genomes of these haploids, apical shoots of all tillers are exposed in an antimicrotubule agent such as colchicine or several types of herbicides. We normally take the young plants from the field, clean them and expose the whole plants in 0.04 % colchicine containing 1.5 % dimethyl sulfoxide (DMSO) solution for 8–24 h at 9–10 °C. After treatment, the plants are washed and transplanted to the field for fertile DH development as shown in Fig. 2.4.

Construction of Chromosome Segment Substitution Lines

The chromosome segment substitution lines are initially derived from F_1 hybrids. Specifically, to obtain more than 100 BC_1F_1 spikes, F_1 is backcrossed to one of its two parents (ideally use 1 ear per F_1 individual for the cross or 2–3 ears if F_1 plants are insufficient) and randomly selected 2–3 BC_1F_1 ears per BC_1F_1 row to produce BC_2F_1 populations next year. In general, the number of backcross generations depends on the specific projects. For example, BC_2F_1 is normally good enough (some projects require BC_3F_1 or BC_4F_1). At BC_2F_1, chromosome component

contributed from the donor parent is about 10 % of the recipient at which time the recombination rate is high and ideal phenotypes or variety can also be selected. The BC_2F_2 lines contain all donor parental genes following a single BC_2F_1 selfing. Generally, BC_2F_4 can be used as a stable population for any research projects related to QTL mapping.

Population Constructions for Fine Mapping

1. Similar to the RIL population, the RHLs are resulted from the F_2's continuous selfing. The uniqueness of RHLs is that one or a few traits from one parent can be maintained, while other loci may be the same as the other parent. Moreover, segregations at all loci examined are always present, resulting in a unique population with the uniform genetic background. The population size can be determined based on the selected traits and data of the observations. For example, evaluation and selection can be initiated at F_3, F_4 or F_5 according to the targeted trait.
2. On the bases of the primary QTL mapping, the QIR population is derived from the backcross to one of parents, and foreground and background selections of different molecular markers based on the major QTLs mapped and the markers located at their flanking sides. For example, we identified the major QTLs for heading and their flanking markers of *Xbarc*320 and *Xwmc*215 and are used for evaluating each backcross population. Meanwhile, genetic background selections are conducted using 200 molecular markers and QTL heterozygotes have now been identified. Noticeably, at least one marker on each chromosome arm should be used during genetic background selection.
3. Similar to QIR construction, SSSLs are the same population as the recipient parent with the exception of the targeted QTL traits contributed by the donor's chromosome segment. This process involves the tracking of the assisted selections on the targeted QTL traits derived from the primary mapping.
4. CSSLs are substitution lines consisting of overlapping chromosome segments which cover the entire genome. They are produced by crossing the multiple donor parents and recipient followed by repeated backcrossing to the latter.

2.1.2.2 Key Notes to Consider During Genetic Population Constructions

Population construction approaches may be different based on different research purposes, and thus some key notes to consider may be different even though they share some in common. Several comments are presented in this section.

1. The two parents of F_1 must be in accordance with the project purpose. In addition, the donor parent (DP) is often from the core germplasm or unique

resource that cannot be used directly, while the recipient parent (RP) represents the best commercial native variety (line).

2. The two parents of F_1 must be highly homozygous; in addition to keep the selected ear, seeds from the remaining ears of the F_1 should be stored as a backup at low temperature, so that it can be used for later propagation after the population is constructed; seeds for recurrent crosses must be from strict selfing (ears must be bagged) of the recurrent parent to avoid false hybrids.
3. While a larger number of F_1 ears are required for F_2 population construction, only 1–2 ears during the initial crosses are needed for other population construction. During this process, strict emasculation of the selected plants must be practiced to avoid self-pollination. Backcross of F_1 generally needs 3–6 ears (using different individuals), whereas BC_1F_1 requires 20 ears (plants). One kg seeds of BC_2F_2 from BC_2F_1 selfing are ideal.
4. For phenotypic evaluation, fertile land should be used for F_2 (BC_2F_2) and F_6 generations, and land for other generations is not critical. Care must be taken when more than one generation/year is practiced using either greenhouse or areas with different climates. Special attention should be taken to prevent loss of some, most, or all of the lines due to undesirable climates or any other bad conditions.
5. To construct near-isogenic lines, backcross lines, or chromosome segment substitution lines, it is the best to use SSRs or biochemical markers for identifying specific genes contributed by the donor parent, thereby speeding up the construction process and improving population qualities.

2.1.3 Quality of Genetic Populations

For QTL mapping, the quality of genetic population includes the population adaptability, population size, homozygosity, and genetic diversity.

2.1.3.1 Parental Selection

Parental selection is closely associated with the adaptabilities of the genetic populations. For example, for grain weight QTL mapping, the two parents must show significant difference in grain size; for Fusarium head blight (FHB) resistance QTL mapping, the two parents must have obvious different resistance to FHB, whereas for quantity trait QTL mappings, it is the best that one parent has the strong gluten, the other has the weak gluten character. It is expected that the larger the differences between the two parents are, the more diversified populations and the better for mapping the traits of interest. Of course, when the two parents show obvious differences in several QTL traits, the resulting populations can be used for their related genetic studies. In general, three principles should be considered for parental

selection. (1) Genetic difference between the parents: the genetic difference between the parents should be neither too large nor too small. Too large difference might affect the hybrid's homologous chromosome pairing, leading to severe reduction of crossovers, thus low recombination rates for loci in the linked segments, biased partitions, and possible hybrid sterility. As a result, the degree of confidence would be dropped dramatically. Too small difference, on the other hand, means low DNA polymorphism and fewer polymorphism markers, resulting in low mapping accuracy. (2) Purity of parents: selfing can guarantee the homozygosity of the parents. (3) Cytological analysis of the parents and their F_1 hybrids: If the parents have been involved in chromosome translocation, chromosome deletion, or being monosomics, they are not ideal for population constructions.

2.1.3.2 Population Size

The mapping population size has a great impact on the accuracy and effectiveness of genetic analysis and QTL detection, especially on QTL detection number, QTL efficacy estimation, and QTL detecting sensitivity (Buckler et al. 2009; Schön et al. 2004; Zou et al. 2005). Xu (1994) found that, with an increase in mapping population size, the estimated likelihood ratio test (LOD) value went up, while both estimated bias of recombination rate and the biased estimation of the QTL genotypic mean and variance decreased. In addition, Beavis (1998) indicated that false positive QTL could still be detected with a population size containing as many as 200 individuals. These studies suggest that constructing a large genetic segregating population is challenging due to the heavy workload and high cost in the process of establishing a genetic linkage map, trait genetic analyses, and QTL detections. Therefore, it is suggested that the size of the population should be determined based on the study purpose. Importantly, the population size should be determined based on the aim of the research. For example, it is estimated that about 200 lines are needed for primary QTL mapping, whereas the population size for fine mapping studies should be as large as possible as the lager the population size, the higher the mapping precision. Additionally, the population size should also be determined by the types of the population being constructed. We believe that F_2 followed by RIL requires a large population in order to allow expressions of all possible genotypes in the population. In general, to achieve high mapping accuracy, the order of population sizes are $F_2 > \text{RIL} > BC_1 > \text{DH}$.

2.1.3.3 Homozygosity and Genetic Diversity

As already discussed, parental homozygosity is critical for constructing highly homozygous populations and elimination of false hybrids is equally important. For populations that need multiple selfing generations, they cannot be used until each line in the population is highly homozygous. Population diversity is closely associated with parental selection.

2.2 Types and Applications of Genetic Markers

Having established various genetic populations, we can now conduct identifications of the genotypes and construct genetic linkage maps using the molecular markers. The advantages of the molecular marker-based detections include the following: (1) the map is presented by DNA sequences; (2) there are a large number of markers distributed in the genome; (3) some markers are highly polymorphic; and (4) some markers are codominant, resulting in intact genetic information. Therefore, molecular markers play important roles in genetic mappings.

Locating QTLs/genes on chromosome(s) can be realized using the four types of genetic markers (morphological markers, cytological markers, biochemical markers, and molecular markers) as discussed below.

2.2.1 Morphological Markers

Morphological markers represent the expressions of plant phenotypic traits that are generated at a specific developmental stage or under a specific environmental condition. Currently, these markers have been widely used in the studies of rice, corn, soybean, wheat, and many others. In wheat studies, for example, researchers have used various aneuploids (e.g., monosomics, nullisomics, trisomics, tetrasomics, and ditelosomics) in mapping some targeted genes on a specific chromosome even on a specific chromosome arm. However, because the use of these types of markers is largely limited by numbers and polymorphisms as well as some of their expressions are affected by gene expression regulation, developmental stage, and environmental factors, this approach has several drawbacks, including that phenotypic differences often do not represent genotypic variations and time-consuming.

2.2.2 Cytological Markers

Cytological markers mainly refer to the chromosome karyotype (i.e., chromosome number, size, centromere position) and bending patterns. These markers have been applied to the mappings of some exogenous genes. To improve wheat agronomic traits, researchers have successfully introduced a number of elite genes into commercial varieties via wide hybridizations and chromosome engineering. The locations of many of these genes/chromosome segments (e.g., C belt, N belt type) have been determined through karyotype and banding pattern analyses. However, the use of these markers has also some limitations, including the following: (1) generation of such resource is difficult, (2) lines with changes in chromosome structures and numbers often show high rate of mortalities due to their poor tolerance to environmental stresses, and (3) fewer number of cytological markers are available.

2.2.3 Biochemical Markers

Biochemical markers refer to the products of gene expressions such as enzymes and proteins (e.g., isozymes and seed storage proteins). To date, there are about more than 180 biochemical markers that have been located on wheat chromosomes, and most of which have been very useful for identifications of exogenous genes in wheat. The use of biochemical marker for identification of wheat high molecular weight glutenin subunits is a great specific example.

2.2.4 DNA Molecular Markers

Rapid development of molecular biotechnologies has made it possible to use the nucleotide sequence variations as genetic markers. The discovery of the restriction fragment length polymorphism (RFLP) reported by Botstein et al. (1980) was considered to be the onset of the use of DNA markers. With the development of (PCR) polymerase chain reaction technology, the second-generation marker systems of random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellite markers (SSR), and sequence characterized amplified region (SCAR) were also developed. Currently, the third-generation marker systems are available, including single-nucleotide polymorphism (SNP) and expressed sequence tag (EST) markers. In recent years, SSR, AFLP, EST, and SNP markers have been widely applied to genetic linkage map constructions, QTL mapping, genetic diversity analyses, and molecular marker-assisted breeding.

2.2.4.1 Restriction Fragment Length Polymorphism (RFLP)

RFLP is useful in revealing the allele variations through autoradiography or non-radioactive graphic techniques. The process involves the digestions of genomic DNA with specific restriction endonucleases, analyses of various DNA fragments separated by agarose gel electrophoresis, and hybridizations with radio- or non-radioactively labeled probes. This system can generate a large number of variants, show codominance and produce intact information, and has a good repeatability and stability. The disadvantages, however, include: it is only useful to analyze the gene rich region; the process is long, tedious, and expensive; and produces lower polymorphism in wheat because this technique is based on southern blot hybridization.

2.2.4.2 RAPD Markers

RAPD is based on PCR (Williams et al. 1990). DNA amplifications are random and polymorphism is generated. Compared with other marker systems, RAPD needs

less DNA, it is easy to operate, and one set of primers can be used in analyzing genomes of different species, it can produce higher polymorphism than RFLP, and ultimately can detect large quantities of genetic polymorphisms rapidly. The issues with this system are as follows: (1) because of its dominant marker nature, it can only be used for dominant analysis; (2) cannot distinguish the homozygous from heterozygous genotypes; (3) cannot provide complete genetic information; and (4) has poor stability and reproducibility. This suggests that the RAPD system is useful for gene mapping, alien chromosome fragment detection, and variety/species diversity studies, but has less value for constructing genetic linkage map in wheat.

2.2.4.3 AFLP Markers

AFLP was first developed by Zabeau and Vos (1993). It combines the advantages of both RFLP and RAPD markers. The digested genomic DNA fragments with the two types of restriction nucleases produce sticky ends which are ligated with the synthetic double-stranded DNA. A subset of the restriction fragments is then selected to be amplified. The amplified fragments are separated and visualized on denaturing polyacrylamide gels, either through autoradiography or fluorescence methodologies, or via automated capillary sequencing instruments. The marker has the advantages of simple and fast, high polymorphism and good stability and therefore can be used to study the genetic diversity of germplasm resources, genetic map construction, and gene mapping. This mark also has dominant nature and shows the DNA fragment length polymorphism. But its wide application has been hampered because of its inability to distinguish DNA of different sequences with the same length.

2.2.4.4 SSR Markers

Simple sequence repeat (SSR) marker is also called the microsatellite marker and has DNA repeat sequences of 1–6 base pairs. The same satellite DNA sequence can be distributed in different genome locations. The polymorphism of SSR comes from random repeat variables in number. Wheat is allohexaploid, and thus its genome size is huge in which approximately 80 % DNA is repetitive. Therefore, the use of SSR markers is more effective in wheat QTL mappings and related studies. Indeed, they have been used in linkage map construction, QTL mapping, and other important QTL locus/gene identifications in various cereal crops. Moreover, because SSR is codominant, it has the ability to detect heterozygote and homozygote. It also provides relative complete genetic information, has good stability and reproducibility, and is easy to use.

2.2.4.5 EST-SSR Markers

Expressed sequence tag (EST) is a gene expression fragment with 300–500 bp. This technology involves in cDNA library construction via mRNA reverse transcriptions, followed by cloning into vectors. The randomly selected clones are sequenced at 5′ or 3′ end, and are compared with the known sequences. Information on species evolution, variation, and senescence can be obtained using this marker system. Therefore, EST-SSR can show the specific gene expression of a specific organ/tissue at a specific developmental stage. The most remarkable feature of EST-SSR is that it can identify functional genes directly as reported in wheat, rice, corn, and other crops.

2.2.4.6 Inter Simple Sequence Repeat (ISSR) Markers

ISSR marker is developed by anchoring 1–4 purine or pyrimidine bases at the 5′ or 3′ end of a SSR sequence and using the anchored bases as primer to amplify a DNA sequence with both ends being reverse complements to the SSR sequence. The polymorphisms of different strains are determined based on the presence or absence of electrophoretic bands. This method is simple, convenient for detection, and repeatable. Therefore, it has been widely applied to gene mapping, species diversity, and systematic developmental studies.

2.2.4.7 Sequence-Character Amplified Regions (SCAR)

SCAR marker is proposed by Paranin et al. in 1993 based on the RAPD technology. Technically, the target DNA sequence can be recovered from the gel after RAPD analysis. The specific primers with 18–24 bases are designed and used to amplify the genome DNA through PCR. Alternatively, inserting about 14 bases at the end of the original primer based on the terminal sequence of the RAPD marker will produce a specific primer that is complementary to the original RAPD marker terminal sequence. Because the SCAR marker is transformed from the RAPD marker, it has a longer primer sequence and thus has a better stability and repeatability than RAPD.

2.2.4.8 Sequence Tagged Sites (STS)

STS primers are RFLP single probe copy probes and microsatellite sequences. Specifically, these primers are developed based on the known probe sequence of both ends of RFLPs. The amplified product is about 200–500 bp DNA sequence occurring only once in the genome and can be used for locating specific gene sites (Olson et al. 1989). Compared with RFLP, STS marker is most advantageous as it does not need to maintain the probe clones, and obtaining the sequence information

from the database is simple and easy. Moreover, the STS marker is codominant and marker information can be shared within genetic profiles of various crops. It is considered to be an intermediary in integrating plant genetic map and physical map. The STS, as a new promising molecular marker, is also codominant, can provide high polymorphism, and generate a great deal of information and therefore has very high application value even though the cost to develop such marker is relatively high.

2.2.4.9 Diversity Arrays Technology (DArT)

Developed by Jaccoud et al. (2001), DArT is a new molecular marker derived from microarray hybridizations that detect the presence versus absence of individual fragments in genomic representations. DArT detects the polymorphism of the DNA fragment harvested from genomic DNA digested by restriction enzymes. The basic principle of DArT is that genomic DNA from different samples is equivalent mixed first, followed by digestion of restriction enzymes, retrieving DNA sections of varying sizes based on electrophoresis, then processing through several steps to produce multiple copies of the smaller fragments, which is called a "representation" and will reduce the complexity of genetic material, and eventually, these fragments are placed as tiny spots onto a batch of identical glass slides using a microarray machine. Each point represents a DNA fragments from a different sample genome, and there are also specific fragment presented only in a few samples. In order to detect the genetic differences among different samples, DArT requires that the "representations" harvested from different samples but digested by the same enzyme are used as probes, to produce corresponding probe combinations which will be used to hybridize with the chip. Since the DNA sequences from different sample are different, the hybrid result for each sample will be different, depending on which spots the DNA binds to, and these differences show the degree of diversity among the samples. These differences can be harvested by machine scanning for signals generated from each DNA spot. In the analysis of polymorphism, the different signals generated from each DNA spot are DArT markers, which can be a representative of a genome polymorphism fragments and can be used as markers for following studies (Jaccoud et al. 2001; Hong et al. 2009).

DArT includes the following steps: the producing of genome fragments using specific methods can reduce complexity of the genome, DArT library construction, chip preparation, sample preparation, chip hybridization, and signal scanning and data processing. The method does not have to know the DNA sequence previously and has the advantage of high quality, high degree of automation, high throughput, and stable results. Although requires a relatively high purity of DNA, DArT has many advantages which enable it applied in genetic linkage map, QTL mapping, identification of germplasm resources, and evolution analysis (Wenzl et al. 2004; Wittenberg et al. 2005; Xia et al. 2005; Akabari et al. 2006).

2.2.4.10 Single-Nucleotide Polymorphism (SNP) Markers

SNP is considered to be one of the third-generation genetic markers and its polymorphism is derived from the change of a single nucleotide in the genome, including single-nucleotide transition, deletion, transversion, and insertion. SNP differences can be identified by analyzing their DNA sequences or alignments of known DNA sequences. The simplicity way is via amplifying a genome segment with specific primers through PCR, followed by PCR product sequencing and alignment. Massive SNP identification can also be achieved by DNA chip technology.

2.3 Statistical and Mapping Method of Quantitative Traits

It is well known that most of the wheat important agronomic and quality traits (e.g., yield, growth cycle, disease/stress resistance, and flour quality) are quantitative traits controlled by polygenic genes (Michelmore et al. 1988). Traditional quantitative genetics neither can determine the number of QTLs controlling these characters, nor can it determine the genetic effects of a single QTL or its location in the chromosome. Researchers often treat the polygenic traits as a whole entity to control a whole group of quantitative traits, and estimate the overall genetic and environmental effects. Although the concept of using genetic markers to detect QTLs had been proposed in 1923 by Sax, few genetic markers for QTL studies were available prior to the 1980s. Due to the rapid development of molecular biology and computer technology since the 1990s, new methods and statistical tools have been developed. The use of these methods and tools, studies on QTL nature, QTL location, and relationship between QTLs and environments have been possible (Paterson 1988; Lander et al. 1989; Dudley et al. 1996; Darvasi et al. 1994).

2.3.1 The Principle of QTL Mapping

QTL mapping is essentially a set of procedure aimed at first detecting and then locating a QTL. It aims to locate the potential genes controlling a quantitative trait using the expected association between the putative genes and the known genetic markers first, and then to estimate their effects.

It is a combination of linkage mapping and quantitative genetics approaches to find an association between genetic marker and a phenotype that one can measure or that can be measured. Therefore, QTL mapping is based on a hypothetical genetic model and a concept of statistics. There are three steps to QTL mapping: (1) linkage map construction; (2) phenotypic evaluation and identification of polymorphism of genetic markers; and (3) statistical analyses to identify and estimate the effect of the loci hat affect the trait(s) of interest.

The essential conditions of the steps are (1) high density linkage map (the average distance between markers should be less than 10 cm) and corresponding statistical analysis method; (2) the target traits can separate in the population and show a continuous pattern (the selected parents should have significant differences and distinct genetic relationship).

2.3.2 Methods of QTL Mapping

With the rapid development of molecular markers, the studies on quantitative traits are now at the QTL mapping stage. QTL mapping and the estimated efficacy value can be realized based on the statistical models used in the QTL mapping. To date, a number of QTL mapping approaches are available, and these include single-interval mapping, composite interval mapping, mixed linear model-based composite interval mapping, inclusive composite interval mapping, as well as the Bayesian analysis.

2.3.2.1 Single-Marker Analysis

Single-marker analysis is used to compare the variations of different QTL genotypic means and identify the relationship between each molecular marker and phenotype based on additive–dominant model, and a variety of statistical analyses, including t-tests, ANOVA, regression, maximum likelihood estimations, and log likelihood ratios. However, the disadvantages of this approach are as follows: (1) it cannot distinguish a single QTL from multiple linked QTLs; (2) cannot estimate the QTL's possible position; (3) QTL effects can be declined due to recombination between the marker and QTL; (4) false positive may be an issue; and (5) requires a large population and has low detection efficiency. Therefore, new model and method are needed for QTL mapping researches.

2.3.2.2 Single-Interval Mapping

The interval (between two adjacent markers) mapping method was then put forward due to the drawbacks of the single-marker analysis. The first model proposed by Jensen was suitable for analyzing DH population and could be used for estimating biased partition. Later, a model suitable for more broad populations was also developed by Knapp et al. (1990). Lander and Botstein (1989) proposed an improved model that based on a maximum likelihood equation with normal mixture distribution and simple regression, which can calculate the LOD score, which indicates the probability that a QTL is present at that position, of any position between two adjusted markers based on genetic linkage maps. LOD scores are plotted along the chromosome map, and those that exceed a threshold significance

level suggest the presence of a QTL in that chromosome region. The most likely QTL position is interpreted to be the point where the peak LOD score occurs. This method can not only determine the possible position QTL in the interval, but also can reduce the size of population required in QTL mapping. In 1992, Haley & Knott and Martinez & Curnow proposed a simple regression interval mapping analysis method. Given the error is independent and followed a normal distribution, the regression analysis and the maximum likelihood ratio test have following relationship, likelihood ratio test UpMSR/MSE $UpF_{regression}$. Linear regression method can obtain the approximate the same result as maximum likelihood method, but has greatly reduced calculation amount, and thus the simple regression method is widely accepted.

There are also some limitations for simple interval mapping. The indicated positions of QTLs are sometimes ambiguous or influenced by other QTLs, and it is difficult to separate effects of linked QTLs. Further, it only uses the information of two markers in each test, while the information of other markers is not fully utilized, and the final mapping results may not be correctly represented.

2.3.2.3 Composite Interval Mapping

In order to overcome the defects of simple interval mapping, Zeng (1994) proposed composite interval mapping (CIM) which combines the multiple regression analysis with interval mapping, and detect QTL in multiple intervals using multiple molecular marker information. The main difference with interval mapping is that the CIM applies multiple regression model in the maximum likelihood analysis which filters out the influence of QTL lying out the interval in the detection of markers at any given point. The basis of this method is an interval test that attempts to separate and isolate individual QTL effects by combining interval mapping with multiple regressions.

The method has the following main advantages: (1) it uses the QTL likelihood map to display the QTL possible positions and significance, thus maintaining the advantages of interval mapping; (2) each time only one interval is examined; (3) estimating of QTL location and effect is asymptotically unbiased if there is no epistasis and QTL–environment interaction; (4) the whole genomic marker information is fully utilized; and (5) with the multiple markers selected, genetic variations generated from other genome regions can be controlled, and mapping accuracy and efficacy can be increased (Zeng 1994).

Disadvantages of this method include the following: (1) it cannot analyze some complex genetic issues, such as epistasis and QTL–environment interaction, and (2) because the intensive computations are used, CIM can be a slow process. Wu et al. (1997) proposed a composite interval mapping method based on the least-square estimation, which is simpler and faster than CIM based on maximum likelihood estimation.

2.3.2.4 Mixed Composite Interval Mapping

Due to the disadvantages of the composite interval mapping, Zhu (1996) proposed the mixed model composite interval mapping which integrates additive, dominant, epistatic and environmental interaction effect into one model. This model assumes (1) a quantitative trait is controlled by multiple QTL genes; (2) the population mean and every major QTL effect (including additive, dominant and epistatic effects) are fixed effects; and (3) the effects of environment, QTL–environment interaction, molecular marker, marker–environment interaction, and the residual error are considered to be random effects. The method combines the effect estimation and QTL location, and is a joint QTL mapping under multiple environments, resulting in a significant improvement in mapping precision and efficiency. The use of the mixed linear model approach in QTL mapping can result in unbiased estimation of QTL–environment effect and has a great deal of flexibility and extensibility. Because this model is based on the composite interval mapping, it can be extended to the analysis of main QTL effects of additive–additive, additive–dominant, dominant–dominant, dominant–epistasis and genetic–environmental interactions. The estimated values of these effects can be used to predict the heterosis of major QTL effect and QTL–environment effect and plant individual breeding value. On the basis of breeding value, individuals with great potential can be selected, and thus the breeding efficiency can be improved. Therefore, the mixed linear model-based composite interval mapping has a greater application prospect. At present, it has been used successfully in the analyses of many important QTL traits that are associated with crop yield and quality.

2.3.2.5 Inclusive Composite Interval Mapping

Composite interval mapping has been widely used in QTL mappings over the past 10 years, yet has some defects in the algorithm. Under the assumption of additivity of QTL effects on the phenotype of a trait of interest, the additive effect of a QTL can be completely absorbed by the two flanking marker variables, and the epistatic effect between two QTL can be completely absorbed by the four marker-pair multiplication variables between the two pairs of flanking markers. For these reasons, Wang et al. (2009) proposed inclusive composite interval mapping (ICIM) method. Two steps are involved in ICIM. In the first step, stepwise regression was applied to identify the most significant regression variables in both cases but with different probability levels of entering and removing variables. In the second step, a one-dimensional scanning or interval mapping was conducted for mapping additive and a two-dimensional scanning was conducted for mapping digenic epistasis. The mapping strategy simplifies the process of background genetic variation controlling in composite interval mapping. The method has low sampling error but high mapping efficiency. When QTL exists, the LOD value is higher; when no QTL exists, the LOD values are close to 0. The interaction of additive QTL of epistasis mapping can be analyzed with this method.

The main advantages of ICIM are as follows: (1) it has a low sampling error, but high mapping efficiency; (2) it has a good robustness for the mapping parameters; (3) epistasis mapping is easy; and in epistasis mapping, it can not only detect the interaction of additive QTLs, but can also detect the interaction of QTLs with no significant additive effects. The disadvantage of this method is that it usually generates too many epistatic loci, resulting in difficulties in the process of selection.

2.4 New Methods of QTL Mapping

As stated above, the development of molecular marker technology since 1980s in the twentieth century has made it possible in the close conjunctions of quantitative traits with the DNA sequence information so that complex QTLs can be mapped at the whole-genome level with the statistical assistance. It provides a great deal to better understand the genetic basis of complex traits and conduct DNA isolation, gene cloning, and marker-assisted breeding. Future research should focus on how the QTL mapping can help reveal the gene dynamic changes during plant development and understand how differences in gene expressions can affect trait development. In recent years, novel QTL analysis approaches and software have emerged (e.g., Fig. 2.5) and is presented below.

2.4.1 Conditional QTL

The recent rapid development of biotechnologies, especially the molecular biology and information technology, brought about a novel concept of studies known as

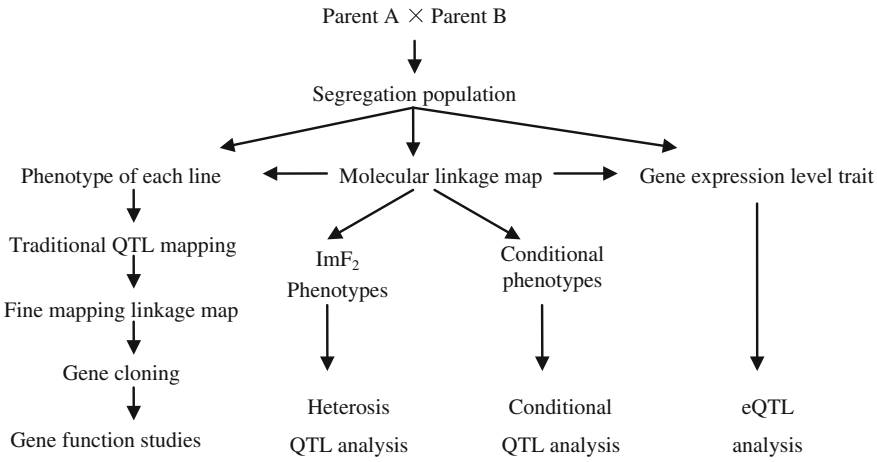

Fig. 2.5 Schematic diagram of several QTL analysis methods

"Conditional QTL." Zhu (1996) published the first paper with such concept in the scientific journal of "Genetics." Soon, Wu et al. (1997) also proposed a method of QTL dynamic mapping and developed a software program to analyze, using the net genetic effect, the QTLs being expressed or silenced during the time period of $t-1$ to t. Since then, a number of traits controlled by conditional QTLs have been analyzed in various organisms, including mice (Atchley et al. 1997), cotton (Ye et al. 2003), and rice (Shi et al. 2001; Guo et al. 2005) using these programs. However, there were no descriptions on the difference between conditional and traditional QTLs previously. Basically, conditional QTL refers to the net inheritable effects resulted from plant growth and development at a specific developmental stage and/or specific agricultural practice under a given condition. It is a method of genetic analysis for analyzing the temporal and spatial expressions and interactive effects of a certain QTLs under various conditions. It can be used to dissect the comprehensive expressions of multiple QTLs during a given organism's (or a specific trait) whole life cycle. Therefore, conditional QTL can be used to study the dynamic development of agronomic traits, QTL response under different agricultural treatments and the cause–effect relationship (see Chap. 1, Volume 2).

For the last ten years, our group focused on the genetic analysis of quality traits with conditional QTL, such as conditional QTL mapping for the dynamic accumulation behavior of grain protein content, for developmental behavior of total starch and its components content, for protein and starch interaction, and for sedimentation volume on seven quality traits (see Chap. 2, Volume 2). In addition, the genetic studies of yield traits with conditional QTL, which include conditional QTL mapping for wheat canopy traits under two different nitrogen application levels, for plant height at different growth stages, for wheat spike dry weight and thousand-kernel weight during different development stages, and for **grain yield and its three components**, etc. **were studied** (see Chap. 3, Volume 2).

2.4.2 eQTL Mapping Method

In 2001, Jansen and Nap proposed the expression QTL (eQTL) mapping method, which uses phenotypic observations, molecular markers, and expression profile data in identification of quantitative trait loci. In the method, the expression level of each gene is treated as a trait, and all the expression information of genes in an individual forms a profile, the linkage of the profile and molecular marker is analyzed. The QTL detected in this method is eQTL (Jansen and Nap 2001). When a gene position is consistent with eQTL-linked marker, the gene underlying the quantitative trait can be determined. The eQTL can be divided into CIS eQTL (Cis-acting eQTL) and trans-eQTL (trans-acting eQTL). CIS eQTL refers to that the eQTL is located to a genome region containing the target gene and indicates that the expression level polymorphism may determine the gene expression difference. An anti-eQTL refers that the eQTL polymorphism underlies the expression difference of a gene located in difference genomic region. A CIS eQTL can directly provide

the information of candidate genes, while a trans-eQTL not only can be combined with other methods to obtain the control network, but also reduce the candidates of the nodes (Rosa et al. 2006). At present, the main method used to study eQTL includes cDNA-AFLP (Vuylsteke et al. 2006), qRT-PCR, and gene chip technology (Potokina et al. 2008). Of these, the gene chip technology has the advantages of high flux, high sensitivity, and is the main approach to study eQTL. The mainly used methods include the following: (1) In transcript-based mapping method, each expression trait (e-trait) is analyzed independently first and then all eQTL of the expression traits are obtained; (2) In marker-based method, the genotype of every marker is identified first and then the expression difference among different marker genotypes was analyzed, testing whether it is associated with the eQTL, and whole-genome-wide scan was performed finally.

eQTL study not only helps us to estimate the heritability of gene expression levels, construct gene regulatory network and mine candidate gene, but also provides conditions for our understanding of gene–gene and gene–environment interactions, so that we can understand the molecular mechanism of biological and genetic basis of complex traits more deeply. eQTL has some shortcomings: (1) the cost is relatively high; (2) improper selection of individuals may lead to partial separation and biased results of QTL mapping; and (3) the current methods for QTL mapping restrict the eQTL study (Liu et al. 2008).

2.4.3 QTL Mapping Methods of New Gene Mining Germplasm

QTL mapping population in crop is generally developed by crossing two homozygous inbreeding lines that often show significant differences. When the two parents carry the same allele, the gene effect cannot be detected even though their effects are large. Therefore, Rao and Xu (1998) proposed a four- even eight-crossway hybridization in order to increase the number of parents. Nevertheless, the parent number is still small. Using statistical techniques, scientists have been able to find novel genes from the germplasm resources, instead of the existing commercial varieties. The QTL statistical method for discovering new genetic resources mainly includes the hitchhiking effect based on association analysis method and a mixed model method based on IBD (Zhang 2006).

Association mapping, also known as "linkage disequilibrium mapping," is a method of mapping QTLs that takes advantage of historic linkage disequilibrium to link phenotypes to genotypes. The primary idea of association mapping is that performing whole-genome scanning and searching for genome segment (or loci) containing selection signal, and then scanning further for important segment and elite alleles (You and Zhang 2007). The main idea of mixed model method based on IBD is that the pedigree is used to compute identical value of the offspring first, and IBD value is embedded in variance component to determine the position and

effect of QTL, and then the best unbiased linear model is used for estimating QTL effect. According to the estimated QTL effect of the varieties, parental selection and molecular design breeding can be carried out, and transferring of genes in varieties can also be studied (Zhang et al. 2005). China is rich in germplasm, and if enough information is obtained, a large number of useful genes can be mined and their effect can be predicted; and the consequent results can be used in molecular design breeding and breeding efficiency improvement (Zhang 2006).

The recent rapid development of crop QTL research progresses has played a significant role in mining new crop gene resources which has benefited the crop genetics and breeding programs. It is imperative to establish efficient QTL mapping methods, including the constructions of new mapping populations, new methods of statistical analysis, and molecular marker-assisted methods for QTL fine mapping, cloning, and selection. This will allow us to better understand crop QTLs and ultimately apply the QTL knowledge to the efficient crop breeding programs. Also, QTL studies will undoubtedly help for the better understanding of gene functions at the whole-genome level and better interpretations of molecular network associated with plant development, interactions between QTLs and environmental changes and biological basis.

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